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EFFECT OF TRANSPORT INHIBITORS ON DIBASIC AMINO ACID EXCHANGE DIFFUSION IN RAT-KIDNEY CORTEX

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SUMMARY

In rat-kidney cortex slices, L-lysine and L-arginine active transport and exchange diffusion, and intracellular Na^+ and K^+ concentrations, have been measured in the presence of temperature reduction, anaerobiosis, 2,4-dinitrophenol, extracellular Na^+ and K^+ deprivation, and ouabain. In general, lysine and arginine influx due to amino acid exchange diffusion appeared to be quantitatively more sensitive than active transport to these inhibitors. These data suggest an effect of these inhibitors at a transport site common to both processes. The most active inhibitors of amino acid exchange also produced marked depletion of intracellular K^+. Restoration of intracellular K^+, however, did not restore amino acid exchange. It is concluded that the changes in cation transport are probably not directly related to the changes in amino acid uptake, but that they represent parallel indices of membrane dysfunction.

INTRODUCTION

The intracellular accumulation of free amino acids against a concentration gradient may occur by active transport or exchange diffusion. Active transport requires metabolic energy for the establishment of a substrate gradient across the cell membrane. Exchange diffusion, on the other hand, has generally been considered independent of metabolic energy, requiring the counterdirectional flow across the membrane of an amino acid which shares a common transport system with the substrate.

In their analysis of glucose transport and exchange diffusion in human red blood cells, ROSENBERG AND WILBRANDT[1,2] proposed that both active transport and exchange diffusion are mediated by the same mobile membrane carrier complex. HEINZ[3] and HEINZ AND WALSH[4] have suggested an analogous model for amino acid

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transport and exchange in Ehrlich ascites tumor cells. HEINZ AND WALSH[4] and JACQUEZ[5] have published graphic representations of this fundamental hypothesis.

This model assumes that the carrier–amino acid complex is diffusable across the cell membrane, and that free carrier is not. On this basis, active uptake is distinguished from exchange diffusion. The former process is thought to require metabolic energy for the migration of free carrier to the transport site. The latter process provides carrier migration in the absence of metabolic energy, in the form of a counterflow amino acid–carrier complex.

JACQUEZ[6,7] has suggested an experimental approach to the testing of some aspects of these assumptions. If metabolic energy is not required for exchange flux, inhibitors of cell metabolism should decrease active transport and not exchange diffusion. JACQUEZ AND SHERMAN[8] have recently extended the preliminary work of JACQUEZ[5,7] in support of this prediction. They measured the effect of several metabolic inhibitors on the transport of amino acids into Ehrlich cells in the presence and absence of counterflow. They found that neither cyanide, 2-deoxyglucose, nor low concentrations of 2,4-dinitrophenol inhibited exchange flux, although all were inhibitors of active amino acid flux.

We have previously presented the exchange diffusion characteristics of lysine, arginine, and ornithine in rat-kidney cortex slices[9]. This communication reports the effect of several metabolic inhibitors, and several inhibitors of cation transport, on the active transport and exchange diffusion of L-lysine and L-arginine in this system.

METHODS

The technique of rat-kidney cortex slice preparation for transport studies has been described[10]. Each sample consisted of there cortex slices, and tissues were paired with regard to donor animal when possible.

All control transport and exchange diffusion studies were carried out in Krebs–Ringer bicarbonate buffer equilibrated with 95% O_2–5% CO_2. Tissues preloaded for exchange were preincubated for 30 min in 2 ml Krebs–Ringer bicarbonate buffer containing 30 mM L-ornithine, L-lysine, or L-arginine (Nutritional Biochemical Corp.), at 37°, pH 7.4. Non-loaded tissues were preincubated without added amino acid. Following the preincubation period, with or without preloading, the slices were blotted on filter paper, and transferred to incubation flasks containing 2 ml Krebs–Ringer bicarbonate buffer with 0.065 mM [^{14}C]lysine or [^{14}C]arginine (New England Nuclear), at 37°, pH 7.4. The uptake of radioactive amino acid was measured according to previously published methods[10] utilizing a boiling-water tissue extract to recover tissue water radioactivity. The distribution of radioactive amino acid into intracellular and extracellular water was calculated after estimation of total tissue water by dessication, and extracellular water by [^{14}C]inulin (New England Nuclear) space[11]. An uptake period of 5 min was used in most of these studies.

Inhibitor studies were performed in one of two ways. In the preliminary studies, tissues were preincubated exactly as controls; that is, in oxygenated Krebs–Ringer bicarbonate buffer ander physiological conditions. Tissues were then transferred to incubation flasks for measurement of uptake of radioactive amino acid at 5 min in the presence of added inhibitor. Except for the temperature reduction experiments, these inhibitor conditions proved inadequate to produce alteration in uptake into preloaded

or non-loaded slices. However, when tissues were preincubated with inhibitor, marked changes occurred. This was accomplished by adding inhibitor to the preincubation buffer used for both preloaded and control tissues. Therefore, slices were exposed to inhibitor during the 30-min preincubation period, and again during the 5-min incubation period. Total water and [^{14}C]inulin spaces were determined to allow for appropriate calculation of uptake under these conditions.

Inhibitors

Temperature reduction. These were the only studies in which preincubation was consistently carried out under control conditions. Tissues were then transferred to Krebs–Ringer bicarbonate buffer, which had been precooled to 21°, and the 5-min uptake at 21° was measured.

Na^+ deprivation. Tris$^+$ was substituted for Na^+ in the Krebs–Ringer bicarbonate formula to provide an isotonic Na^+-free buffer (pH 7.4). Bicarbonate was omitted from this buffer, and the solution was gassed with 100% O_2.

Anaerobiosis. Krebs–Ringer bicarbonate buffer was equilibrated with 95% N_2–5% CO_2, and preincubation and incubation flasks were thoroughly gassed with 95% N_2–5% CO_2 to ensure anaerobic conditions.

2,4-Dinitrophenol (Fisher). Krebs–Ringer bicarbonate buffer equilibrated with 95% O_2–5% CO_2 was used, and dinitrophenol was added to provide a concentration of 0.1 mM.

Ouabain (Z. D. Gilman, Inc.). 0.8 mM was added to oxygenated Krebs–Ringer bicarbonate buffer.

K^+ deprivation. NaCl and NaH_2PO_4 were substituted for the potassium salts in the Krebs–Ringer formula.

For measurement of tissue cation content, slices were extracted with 5 ml 0.5 M H_2SO_4 for 20 min in a boiling-water bath. The extract was cleared by centrifugation, and an aliquot prepared with Li_2SO_4. Na^+ and K^+ concentrations were determined in a flame photometer (National Instrument Company) using a Li_2SO_4 internal standard. Extracellular water Na^+ and K^+ were obtained by measurement of the buffer cation concentrations.

Expression of data

Tissue Na^+ and K^+ content are expressed as mequiv/l intracellular water, by difference between total tissue water concentration and extracellular water concentration.

Radioactive amino acid uptake data are expressed as mμmoles of amino acid accumulated per ml intracellular water during the 5-min incubation. This was calculated from the amino acid distribution ratio, (counts/min per ml intracellular water)/(counts/min per ml extracellular water) multiplied by the extracellular amino acid concentration, 0.065 mM. The following symbols are used to simplify tabular presentation: F = uptake of amino acid into preloaded cells under control conditions; F_i = uptake into preloaded cells in the presence of inhibitor; T = uptake into non-loaded cells, control conditions; T_i = uptake into non-loaded cells, inhibitor added. Quantities of F, F_i, T and T_i are presented as mμmoles $\pm$ standard deviation.

From these data several derived values have been obtained. Net influx due to exchange diffusion is determined by difference between uptake into preloaded and

non-loaded cells, $X = F-T$. Exchange flux in the presence of inhibitor, $X_i = F_i-T_i$. For comparison of the overall effect of inhibitor on uptake into preloaded *vs.* non-loaded tissues, the differences $\Delta F = F-F_i$, and $\Delta T = T-T_i$ are presented. Because uptake has been measured under paired tissue conditions, quantities for X, X_i, ΔF, and ΔT have been calculated as the mean of the differences of each individual study.

RESULTS

Tissue water spaces

The total tissue water of kidney slices incubated for 30 min in oxygenated Krebs–Ringer bicarbonate buffer is 80%, and this figure has been used for calculation of Na^+ and K^+ concentration, and amino acid flux under control conditions. Total tissue water measured after 30 min in anaerobic Krebs–Ringer bicarbonate, Na^+-free Tris buffer, and dinitrophenol was 84%, 77% and 82%, respectively, of wet tissue weight.

The extracellular distribution space of [^{14}C]inulin was measured at 5 and 10 min for calculation of transport fluxes. These values were 17% and 20%, respectively, of wet tissue weight. 30-min values were obtained in the presence and absence of inhibitors. The usual extracellular fluid of kidney slices incubated for 30 min in oxygenated Krebs–Ringer bicarbonate is 26% of wet tissue weight. The following values were observed under inhibitor conditions: anaerobic Krebs–Ringer bicarbonate, 31%; Na^+-free, 20%; dinitrophenol, 25%. In the estimation of intracellular water, both anaerobic and Na^+-free media produced significant changes in the total water—extra cellular fluid difference. The appropriate values were used for calculation of the inhibitor results.

Preloading with 30 mM lysine or arginine did not change tissue water or extracellular water values.

Effect of temperature reduction

The uptake of both [^{14}C]lysine and [^{14}C]arginine was significantly reduced during the 5-min incubation at 21° (Table I, T *vs.* T_i). A significant inhibition of uptake was also observed for both amino acids into tissues preloaded with lysine, arginine, or ornithine (F *vs.* F_i). The exchange uptake of lysine was not changed at reduced temperature (X *vs.* X_i). However, the exchange uptake of arginine was markedly inhibited at 21°.

The transport and exchange model predicts that $X = X_i$ or $\Delta F = \Delta T$. This prediction is met by the data for lysine uptake. The arginine studies, on the other hand, are not compatible with the model according to the assumptions of this experimental design. For arginine transport, $X > X_i$, and $\Delta F > \Delta T$.

Na^+ deprivation

Na^+-free buffer did not inhibit the uptake of lysine or arginine into non-loaded or preloaded cells, when tissues were preincubated in Krebs–Ringer bicarbonate buffer. Preincubation with Na^+-free buffer produced inhibition of arginine uptake into unloaded tissues; lysine uptake was not significantly reduced (Table II, T *vs.* T_i). In preloaded tissues, Na^+ deprivation led to marked inhibition of exchange uptake of lysine into both arginine and lysine preloaded tissues; arginine exchange was marked-

TABLE I

INHIBITION OF TRANSPORT AT REDUCED TEMPERATURE (21°)

Values are expressed as mμmoles of amino acid accumulated per ml intracellular water in 5 min ± standard deviation (n = 4–6). See METHODS for definition of symbols.

Amino acid		F	T	F_i	T_i	X	X_i	ΔF	ΔT
Preloaded	*Exchanged*								
Lys	Lys	181 ± 20	72 ± 27	149 ± 27	38 ± 14	110	111	34	34
Arg	Lys	128 ± 14	71 ± 17	103 ± 23	31 ± 4	56	71	39	41
Orn	Lys	212 ± 64	70 ± 17	157 ± 23	31 ± 14	141	126	55	42
Lys	Arg	210 ± 46	81 ± 14	107 ± 25	51 ± 14	130	49*	58	29**
Arg	Arg	166 ± 37	81 ± 14	101 ± 23	51 ± 14	72	49*	63	29**
Orn	Arg	250 ± 51	77 ± 14	113 ± 23	47 ± 17	153	65*	113	38**

* $P < 0.05$ when compared with corresponding value of X.
** $P < 0.05$ when compared with corresponding value of ΔF.

ly reduced into lysine but not arginine preloaded tissues (X *vs.* X_i). Therefore, for three of the four studies, $X > X_i$, and $\Delta F > \Delta T$.

Anaerobiosis

A 5-min anaerobic incubation did not alter uptake or exchange into tissue preincubated aerobically. Anaerobic preincubation, however, resulted in almost complete inhibition of exchange uptake in each instance (Table III, X_i). In non-loaded cells, only arginine uptake was significantly reduced by an aerobiosis. For all of the studies, $X > X_i$, and $\Delta F > \Delta T$.

2,4-Dinitrophenol

Dinitrophenol was not inhibitory in tissues preincubated in Krebs–Ringer bicarbonate buffer without dinitrophenol. Addition of 0.1 mM dinitrophenol to preincubation and incubation flasks produced the changes shown in Table III. Arginine uptake into non-loaded tissues, but no lysine, was inhibited. Uptake into preloaded

TABLE II

INHIBITION OF TRANSPORT BY Na^+ DEPRIVATION

Conditions are described in the text. Values are expressed as mμmoles of amino acid accumulated per ml intracellular water in 5 min ± standard deviation (n = 4–6). See METHODS for definition of symbols (n = 4).

Amino acid		F	T	F_i	T_i	X	X_i	ΔF	ΔT
Preloaded	*Exchanged*								
Lys	Lys	170 ± 32	69 ± 19	112 ± 7	73 ± 15	87	40*	59	0**
Arg	Lys	176 ± 8	63 ± 19	105 ± 16	84 ± 10	98	24*	71	0**
Arg	Arg	158 ± 8	99 ± 9	118 ± 23	58 ± 2	58	50	39	31
Lys	Arg	230 ± 32	91 ± 13	93 ± 13	72 ± 8	144	25*	138	30**

* $P < 0.05$ when compared with corresponding value of X.
** $P < 0.05$ when compared with corresponding value of ΔF.

TABLE III

EFFECT ON TRANSPORT OF ANAEROBIOSIS, 2,4-DINITROPHENOL, OUABAIN, AND K^+-FREE MEDIA

Conditions and symbols described in the text ($n = 4$).

Amino acid		*Inhibitor*	X	X_t	ΔF	ΔT
Preloaded	*Exchanged*					
Lys	Lys	N_2	190	43*	148	17**
Arg	Lys		78	0*	40	8**
Lys	Arg		151	0*	151	33**
Arg	Arg		61	0*	101	36**
Lys	Lys	Dinitrophenol	136	78*	56	0**
Arg	Lys		78	67	20	0**
Lys	Arg		122	56*	96	31**
Arg	Arg		47	36	38	27
Lys	Lys	Ouabain	127	154	0	0
Arg	Lys		78	74	0	0
Cys	Arg		157	188	0	0
Arg	Arg		81	84	0	0
Arg	Lys	K^+-free	48	48	0	0
Arg	Arg		51	56	0	0

* $P < 0.05$ when compared with corresponding value of X.
** $P < 0.05$ when compared with corresponding value of ΔF.

cells revealed a phenomenon which had not been noted in other inhibitor studies. Tissues varied not according to the radioactive amino acid being studied, as in the temperature studies, but rather, according to the preloaded amino acid. This finding led to the re-evaluation of the preincubation period described later.

Ouabain

There was no significant inhibition of uptake into preloaded or non-loaded cells when 0.8 mM ouabain was added to the preincubation and incubation media (Table III).

K^+ deprivation

Two screening studies revealed no effect of a K^+-free buffer on active transport or exchange diffusion.

Effect of inhibitor preincubation on preloading of lysine and arginine

In ascites tumor cells, HEINZ AND WALSH[4] have demonstrated that the stimulation of uptake induced by counterflow is directly related to the concentration gradient established for the counterflow amino acid. We have shown a similar relationship for lysine autoexchange diffusion in the rat-kidney cortex[9]. The dinitrophenol results for lysine preloading suggested that loading might be altered by the inhibitor. This was not initially anticipated since the kinetics of the previously studied dibasic system of this tissue predicted that passive diffusion with intracellular fluid–extracellular fluid equilibration should occur at a 30 mM concentration[12].

When the loading of 30 mM arginine was studied with [^{14}C]arginine, an amino acid distribution ratio of 1.00 ± 0.14 was attained at 30 min; 30 mM lysine uptake,

however, reached a ratio of 1.77±0.22 at 30 min. This intracellular concentration of lysine, despite a high incubation level of lysine, has led to the definition of what appears to be a high-K_m transport system for lysine[13].

The effect of metabolic inhibitors on the preloading phase was examined. Small changes, not statistically significant, were observed for 30 mM arginine loading. If the control distribution ratio is considered 100%, the ratios in the presence of inhibitors were as follows: anaerobiosis, 85%; Na^+-free, 100%; dinitrophenol, 90%. Major changes were observed for 30 mM lysine loading. Assigning 100% for the 30-min value of 1.77, inhibitors were as follows: anaerobiosis, 60%; Na^+-free, 60%; dinitrophenol, 70%.

The minor restriction of arginine preloading cannot completely explain the marked inhibition of exchange found anaerobically, and in one case with Na^+-free media. The minimal changes induced by dinitrophenol in arginine-preloaded cells could be on this basis, however.

Lysine loading at 30 mM is both concentrative, and sensitive to metabolic inhibitors. The intracellular lysine concentration obtained when loading is carried out under control conditions is 50 mM. When preloading is performed anaerobically, in the absence of Na^+, or with dinitrophenol, the intracellular concentration is more nearly 30 mM. This difference may be responsible for a significant decrease in exchange diffusion. An estimate of the degree of decrease may be obtained from data presented in another publication[9] in which lower levels of preloaded lysine were established by varying the preloading concentration. A decrease from 50 mM to 30 mM intracellular lysine should result in a decrease in lysine uptake of 20–25%. Thus, X_i could be reduced to 75% of X on the basis of decreased lysine preloading in the presence of anaerobiosis, Na^+-free media, or dinitrophenol. The experimental findings, however, indicated X_i to be less than 75% of X in most of these studies.

Effect of inhibitor preincubation on cation transport

The inhibitors were introduced in order to evaluate the dependence of amino acid transport on cell metabolism. In practice, rather severe conditions of inhibitor preincubation were required, conditions which may produce widespread tissue damage. As an indicator of tissue injury we elected to study the alterations of intracellular Na^+ and K^+ caused by a 30-min incubation with inhibitor.

The data are summarized in Table IV. The values presented for tissues examined immediately after slicing, without incubation, were calculated using the tissue water spaces of slices incubated 30 min in oxygenated Krebs–Ringer bicarbonate buffer. After incubation in aerobic Krebs–Ringer bicarbonate buffer for 30 min, an intracellular fluid/extracellular fluid concentration gradient of K^+ was preserved although there was a significant shift of Na^+ and K^+ during this period. Preloading with 30 mM lysine or arginine in Krebs–Ringer bicarbonate buffer produced only small differences, significantly different in only one case when compared with unloaded tissues. (In lysine-loaded cells, $[Na^+] = 97\pm4$, $[K^+] = 73\pm9$ ($P<0.05$); in arginine-loaded cells, $[Na^+] = 96\pm12$, $[K^+] = 79\pm7$.)

Incubation with inhibitors produced marked alterations in tissue cation content. With one exception, these changes were identical in non-loaded and preloaded cells. (In Na^+-free media, intracellular $[K^+]$ was significantly lower ($P<0.05$) in arginine-preloaded cells, 36 ± 7; in lysine-loaded cells the intracellular $[K^+]$ was

TABLE IV

EFFECT OF INHIBITORS ON INTRACELLULAR Na^+ AND K^+ CONCENTRATIONS

Tissues were incubated with inhibitor for 30 min as described in the text. Data are expressed as mequiv/l intracellular water ± standard deviation (n = 4–12).

Conditions	*Intracellular*	
	[Na⁺]	*[K⁺]*
No incubation	42 ± 7	144 ± 1
Oxygenated Krebs–Ringer bicarbonate	88 ± 13	87 ± 8
Anaerobic Krebs–Ringer bicarbonate	155 ± 5	22 ± 2
Na^+-free	16 ± 4	47 ± 3
Dinitrophenol (0.1 mM)	118 ± 9	53 ± 4
Ouabain (0.8 mM)	111 ± 6	56 ± 4
K^+-free	102 ± 10	54 ± 4

44±3.) These data suggest a correlation between the degree of reduction of intracellular K^+, and the degree of inhibition of amino acid exchange diffusion induced by each inhibitor. Anaerobiosis, which completely abolished exchange diffusion in most cases, also exerted the most pronounced effect on cation transport. The next most potent exchange inhibitor was Na^+-free buffer which reduced intracellular K^+ to the range of 27–47 mequiv/l in preloaded tissues.

Recovery of tissue treated anaerobically

Because the 30-min anaerobic incubation produced such profound shifts of Na^+ and K^+, and such profound inhibition of a supposed passive cell process, amino acid exchange diffusion, the viability of these tissues was questioned. WHITTAM AND

TABLE V

RECOVERY OF TISSUES PREINCUBATED ANAEROBICALLY

Tissues were preincubated anaerobically for 30 min and then incubated aerobically for 5 or 10 min, as indicated. In the high-K^+ studies, after anaerobic preincubation, tissues were incubated aerobically for 10 min in an isotonic Ringer buffer modified to contain 50 mequiv/l of K^+.

Auto-exchange	*Time (min)*	*Preloading* buffer*	*Incubation* buffer*	*(mμmoles uptake per 5 min)*			*Intracellular (mequiv/l)*	
				F	*T*	*X*	*[Na⁺]*	*[K⁺]*
Lysine	5	KRB–O_2	KRB–O_2	210	82	128	96	80
		KRB–N_2	KRB–N_2	107	65	43	151	20
		KRB–N_2	KRB–O_2	115	67	44	132	39
Arginine	10	KRB–O_2	KRB–O_2	252	142	117	97	84
		KRB–N_2	KRB–N_2				155	22
		KRB–N_2	KRB–O_2	103	106	0	133	44
		KRB–O_2	High K^+	232	121	110	38	140
		KRB–N_2	High K^+	109	125	0	83	92

* KRB–O_2, Krebs–Ringer bicarbonate buffer equilibrated with 95% O_2–5% CO_2; KRB–N_2, Krebs–Ringer bicarbonate buffer equilibrated with 95% N_2–5% CO_2.

DAVIES[14] have shown that guinea kidney slices preincubated anaerobically for 15 min exhibit active cation transport when transferred to an aerobic medium.

This was tested (Table V) by transferring 30-min anaerobically preincubated slices to Krebs–Ringer bicarbonate buffer equilibrated with 95% O_2–5% CO_2. There were changes of intracellular [Na^+] and [K^+] toward normal after 5 and 10 min of incubation. Determination of transport and exchange of amino acid into these recovering slices revealed that although active cation pump function was occurring, exchange diffusion was not restored.

Another recovery experiment (Table V) was devised to test whether the low intracellular K^+ was causally related to the reduction in exchange diffusion. In this study, after a 30-min anaerobic preincubation, tissues were transferred and preloaded in an aerobic Ringer bicarbonate buffer containing K^+ (50 mequiv/l) which had been substituted for an equivalent concentration of Na^+. Arginine autoexchange diffusion was measured for 10 min. The high-K^+ buffer restored tissue Na^+ and K^+ levels to normal values, and of itself was not an inhibitor of exchange diffusion. However, despite a normal intracellular [K^+], exchange diffusion was not restored. It seems unlikely, therefore, that the low intracellular [K^+] of anaerobically preincubated tissues is the direct cause of the exchange diffusion inhibition.

DISCUSSION

The temperature studies provided the least complex experimental system in that the preloading phase was identical for control and inhibitor conditions. The results of the arginine transport studies were distinctly different from those with lysine, and no explanation for this difference is apparent.

The other inhibitor studies, in general, revealed greater inhibition of exchange flux than of active transport flux. The conditions for inhibition were quite severe, however, as indicated by the result of the tissue Na^+ and K^+ measurements. When severe inhibition conditions were applied by JACQUEZ AND SHERMAN[8] in the form of high dinitrophenol concentrations, similar results were obtained.

Inhibition of exchange diffusion has previously been reported by JOHNSTONE AND QUASTEL[15] and JOHNSTONE AND SCHOLEFIELD[16]. They reported a marked inhibition of exchange flux in ascites tumor cells incubated with tribromoethanol, indole, and to a lesser extent, pentothal. These compounds were also shown to inhibit active transport flux without interfering with cell metabolism. More recently, OXENDER AND WHITMORE[17] presented an abstract in which they describe similar effects of tribromethanol and dinitrofluorobenzene on amino acid exchange in ascites cells, and minimal effects on active uptake. However, although JOHNSTONE and her co-workers interpreted their data in support of a common site for active and exchange flux, OXENDER AND WHITMORE have suggested the possibility of separate sites.

In the kidney cortex we have observed several types of response to inhibitors. Variation of results due to temperature reduction have been stated. In Na^+-free media the lysine uptake studies revealed exchange inhibition without active transport inhibition. Although in anaerobic media the results theoretically were those predicted for tissues which had undergone complete cellular disorganization, these tissues retained the ability to restore cation transport, but not amino acid exchange diffusion, when reoxygenated. Although Na^+ dependence of exchange was apparent, the cation

pump could not be directly linked to the exchange process for dibasic amino acids. Thus, ouabain did not alter exchange of amino acids; there was not a mole–mole exchange of lysine or arginine for K^+; restoration of intracellular K^+ did not restore amino acid exchange.

In these studies, amino acid exchange was inhibited to a greater extent than active transport. In terms of the active transport and exchange model, this could be explained by inactivation of carrier transport sites which are common to both processes. If such sites are rapid turnover membrane units requiring cell metabolism for continued replacement of functional components, then prolonged preincubation with inhibitor might result in the disruption of cell membrane transport function.

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