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COMPETITIVE STIMULATION: FURTHER EVIDENCE FOR TWO CARRIERS IN THE TRANSPORT OF NEUTRAL AMINO ACIDS

JOHN A. JACQUEZ

Departments of Biostatistics and Physiology, The University of Michigan, Ann Arbor, Mich. (U.S.A.)

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SUMMARY

1. Two explanations have been proposed for the stimulation of uptake of one amino acid in the presence of another, a phenomenon which has been demonstrated for some pairs of amino acids in uptake studies with ascites tumor cells. One explanation invokes one carrier transport system and an exchange reaction at the outer surface of the cell. The other requires the presence of two carrier transport systems, a rapid uptake of one amino acid by one system and an exchange counterflow mediated by the other system.

2. Recently INUI AND CHRISTENSEN[9] have presented strong evidence for the presence of two systems, one requiring Na^+.

3. In the experiments reported here it has been found that omission of Na^+ from the medium eliminates the stimulation of uptake of tryptophan by methionine in Ehrlich ascites cells. This supports the second of the above hypotheses.

INTRODUCTION

A number of laboratories[1-5] have reported that the uptake of some amino acids by ascites tumor cells is stimulated in the presence of certain other amino acids and that the maximal stimulation is obtained when the two amino acids are present in approximately equimolar amounts. This phenomenon has been named 'competitive stimulation'. Recently SCHAFER AND JACQUEZ[6] have measured the simultaneous uptakes in Ehrlich ascites cells of each of the amino acids of a number of pairs showing competitive stimulation. They found that while the uptake of one of the amino acids was increased, that of the other was always decreased and that the decrease in uptake of the inhibited amino acid was in each case considerably greater than the increase in uptake of the stimulated amino acid.

Two explanations have been offered for the phenomenon of competitive stimulation. On the basis of studies of the mutual inhibitory effects of the amino acids, OXENDER AND CHRISTENSEN[7] proposed that there were two transport systems for the neutral amino acids which they designated the A and L systems. They defined the A system as a poorly exchanging system which serves as the primary system for

transport of amino acids such as alanine, proline, α-aminoisobutyric acid and glycine and the L system as a rapidly exchanging system which has high affinity for the long-chain and aromatic amino acids. They suggested that in competitive stimulation one of the amino acids is transported rapidly by the A system and then stimulates the uptake of the other amino acid by an exchange mediated counterflow. On the other hand, GUROFF, FANNING AND CHIRIGOS[5] suggested that an exchange reaction at the outer surface of the cell might be involved. For this explanation to hold one would have to assume that the stimulated amino acids bind to the carrier quite slowly but that the other amino acid binds to the carrier rapidly and that the exchange reaction between this latter carrier–amino acid complex and the free amino acid whose uptake is stimulated is rapid. I have shown that a model of carrier transport which incorporates these assumptions can predict the phenomenon of competitive stimulation and until recently have favored this interpretation[8]. However, INUI AND CHRISTENSEN[9] have recently shown that the portion of the transport flux of L-methionine which they attribute to the A system on the basis of its inhibition by α-aminoisobutyric acid is dependent on the presence of Na^+ and disappears in the absence of extracellular Na^+. When considered in conjunction with the experiments of JOHNSTONE AND SCHOLEFIELD[10] which showed that the exchange flux of methionine is independent of extracellular Na^+ this is strong evidence for the existence of two distinct carrier systems, one requiring Na^+, the other not requiring Na^+. This information clearly provides the basis for designing an experiment which is critical in one direction at least. On the hypothesis of OXENDER AND CHRISTENSEN the stimulation of uptake seen in experiments on competitive stimulation must disappear if Na^+ is omitted from the extracellular medium. If it does not their explanation is invalid; if it does we have strong support for their suggestion, although not conclusive support since we do not yet have an experimental manipulation which will allow us to carry out the inverse experiment, that is we cannot inactivate the L system and leave the A system intact. In this paper I report the results of a number of such experiments. As will be seen in the data the results support OXENDER AND CHRISTENSEN.

MATERIALS AND METHODS

The Ehrlich ascites cells used in these experiments were from a hypotetraploid line carried in this laboratory by weekly intraperitoneal inoculation of 0.1 ml of ascites, diluted 2:1 with sterile saline, into female Swiss albino mice.

Salt solutions

Five modifications of Krebs–Ringer phosphate have been used in these experiments, three of them free of Na^+. The solution in routine use in this laboratory is made up by adding 10 ml of a phosphate buffer solution (pH 7.0) to 46.5 ml of a standard salt solution. The salt solution is made up to contain 144 mM NaCl, 5.76 mM KCl, 1.03 mM $CaCl_2$, 1.44 mM KH_2PO_4 and 1.44 mM $MgSO_4$. The phosphate buffer is made up from stock solutions of 154 mM NaH_2PO_4 and 100 mM Na_2HPO_4. This solution will be referred to as Na-KRP solution (1). The other Na^+-containing solution was made up with the same standard salt solution but the buffer solution was made up from stock solutions of 154 mM KH_2PO_4 and 100 mM K_2HPO_4. This

will be referred to as Na-KRP solution (2). For the other three solutions potassium phosphates were also used to make up the buffer solutions. For these the NaCl was replaced by an osmotically equivalent amount of choline chloride, sucrose, or KCl. These are designated Ch-KRP, S-KRP and K-KRP solution, respectively, in the remainder of this paper.

Collection and preparation of ascites cells

The methods of preparing the cells for experiments differ somewhat from one laboratory to another. The major variations are in the use or non-use of osmotic shock and in the temperature at which the preliminary washings are carried out. The experiments to be described were run with and without the use of osmotic shock and with the collection and washing procedures carried out both at room temperature (20–25°) and at cold-room temperatures (2–5°). Mice bearing 6–7-day-old ascites were killed by dislocating the cervical spine, the abdomens were opened, and the ascites removed with a pipette. In 12 experiments, 20–50 ml of ascitic fluid were added to 50 ml of Na-KRP solution (1) or Na-KRP solution (2) (pH 7.0) containing 0.2 mg heparin. If osmotic shock was used the suspension was diluted with an equal volume of distilled water, was filtered through cheese cloth and centrifuged. When osmotic shock was not used the dilution was with Na-KRP solution (1). In two additional experiments in which osmotic shock was used the cells were collected into K-KRP solution (pH 7.0). In the 12 experiments mentioned above the cells were resuspended in the same Na-KRP solution as was used for the initial collection of the cells, in the other two they were resuspended in K-KRP solution. The suspension was then divided into three equal parts, each of which was centrifuged and washed and finally resuspended in one of the salt solutions, Na-KRP (1), Na-KRP (2), Ch-KRP, S-KRP or K-KRP.

Experimental procedure

The unlabeled L-methionine and L-tryptophan were of grade A from California Biochemicals. The labeled L-tryptophan was L-[3-^{14}C]tryptophan from the New England Nuclear Corp.

RESULTS AND DISCUSSION

The results are summarized in Table I which gives the distribution ratios for L-tryptophan after 1, 2 or 10 min of incubation. Each number is the mean of two replicates; the coefficients of variation (standard deviation of mean divided by the mean) had a mean value of 0.02 and a range of 0.005 to 0.054. The uptake of L-tryptophan is decreased in the cells treated by osmotic shock, the effect being very marked in those cells treated with osmotic shock and the washing procedure at room temperature. I have not encountered such marked effects previously and suspect that it is related to the long period of handling and repeated washing superimposed on the osmotic shock. As can be seen from the 2-min incubations, there were no differences qualitatively between the results obtained with the use of Na-KRP solution (1) and (2). In all experiments, the uptake of tryptophan was markedly decreased when Na^+ was omitted from the medium. However, the significant finding is that the uptake of L-tryptophan was stimulated in the presence of L-methionine in all ex-

TABLE I

EFFECT OF L-METHIONINE ON UPTAKE OF L-TRYPTOPHAN IN PRESENCE AND ABSENCE OF Na^+

Expt. No.	*Osmotic shock**	*Temp. of washes***	*Na-KRP****		*Ch-KRP*		*S-KRP*		*K-KRP*	
			T§	*TM*§	*T*	*TM*	*T*	*TM*	*T*	*TM*
1 min incubation										
1	No	R	2.12	3.27	0.98	0.81	0.95	0.86	—	—
2	No	R	2.22	3.16	1.25	0.95	—	—	0.91	0.65
3	Na-KRP (1)	R	0.81	1.68	0.37	0.53	0.38	0.49	—	—
4	Na-KRP (1)	C	1.95	2.49	1.38	1.20	—	—	0.97	0.71
5	K-KRP	R	0.76	1.64	0.40	0.52	0.44	0.50	—	—
6	K-KRP	C	1.91	2.74	1.38	0.94	—	—	0.84	0.55
2 min incubation										
7	No	R	3.12	5.32	1.76	1.26	1.61	1.17	—	—
8	No	C	3.28	4.91	2.47	2.14	—	—	1.23	0.87
9	Na-KRP (1)	R	1.54	3.70	0.75	0.96	0.74	0.82	—	—
10	Na-KRP (1)	C	2.00	2.95	1.16	1.01	—	—	0.80	0.53
11	Na-KRP (2)	R	1.05	2.97	0.79	0.94	0.66	0.78	—	—
12	Na-KRP (2)	C	2.09	2.80	1.69	1.24	2.33	1.82	—	—
10 min incubation										
13	Na-KRP (2)	R	2.14	4.74	1.17	1.44	1.08	1.07	—	—
14	Na-KRP (2)	C	4.12	6.54	2.43	2.00	2.50	2.05	—	—

* No, no osmotic shock; Na-KRP, osmotic shock with half-isotonic Na-KRP solution; K-KRP, osmotic shock with half-isotonic K-KRP solution.

** R, washes at room temperature (20–25°); C, washes carried out in cold room (2–5°).

*** Suspending medium. For the Na^+-containing media, the medium used was the same as the one used for the osmotic shock, *i.e.* Na-KRP solution (1) or (2).

§ Distribution ratio for L-tryptophan for cells in 1 mM L-tryptophan (T) or in a mixture of 1 mM L-tryptophan and 1 mM L-methionine (TM).

periments in which Na^+ was present and was inhibited in the presence of L-methionine in all except Expts. 3, 5, 9, 11 and 13, and even in these experiments the remaining effect was very small. In Expts. 3, 5, 9 and 11 the uptake of tryptophan was slightly larger in the presence of methionine in Ch-KRP and S-KRP solution; in Expt. 13 this small effect was present with Ch-KRP but not with S-KRP solution. The common feature shared by these five experiments is that all were on cells which had been subjected to osmotic shock and the washing procedures at room temperature and that even in the presence of Na^+ the uptake of tryptophan in these cells was much lower than in cells which had not been osmotically shocked or which had been so treated at cold-room temperatures. Although a small effect remained in these experiments this should not obscure the fact that there was nonetheless a marked decrease in the effect of methionine in the absence of extracellular Na^+. Furthermore in the first four of these experiments the distribution ratios are below 1 in the absence of Na^+ and in Expt. 13 it barely rises above 1. The results of the other experiments in which the cells are obviously in good condition are so clearcut that I would discount the small effect remaining in Expts. 3, 5, 9, 11 and 13 as being related somehow to the poor condition of the cells and not directly to the effect under investigation.

In conclusion, in nine experiments in which the cells were in good condition, as measured by their ability to transport tryptophan in the absence of methionine, the

effect of methionine was to stimulate uptake in the presence of Na^+ and to inhibit it in the absence of Na^+. In five experiments in which the cells were in poor condition the stimulatory effect of methionine was markedly decreased when Na^+ was omitted from the medium but a small effect did remain. However, the residual effect did not involve stimulation of uptake of L-tryptophan against the gradient. All in all these experiments strongly support the interpretation of OXENDER AND CHRISTENSEN to the effect that competitive stimulation represents a rapid uptake of one amino acid by a Na^+-dependent transport system followed by an exchange counterflow of the other amino acid mediated by another transport system. Possibly these two systems do not represent distinct carrier species. Perhaps an explanation of the type postulated by CRANE, FORSTNER AND EICHHOLZ[12] to explain the effect of Na^+ on sugar transport in the intestine is applicable, that is, the carrier–amino acid complex can cross the membrane with or without Na^+ bound to the carrier and these two forms of the carrier have different affinities for the same amino acid.

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