PRELIMINARY NOTES

Change in Na+ uptake during amino acid transport

In some of their early studies, CHRISTENSEN et al.[1,2] found that a net efflux of K+, and a partial replacement of intracellular K+ by Na+, accompanied uptake of amino acids by Ehrlich ascites cells and that concentrative uptake was inhibited by the replacement of extracellular Na+ by K+ or choline. Subsequently RIGGS, WALKER AND CHRISTENSEN[3] suggested that uptake of amino acids by Ehrlich ascites cells depended on intracellular K+ levels. However, the results of HEMPLING AND HARE[4] did not fit this hypothesis and KROMPHARDT et al.[5] found that glycine uptake depended on extracellular Na+, not intracellular K+. WHEELER et al.[6] have reevaluated the work of RIGGS, WALKER AND CHRISTENSEN[3] and now conclude that the results actually gave evidence for a dependence of amino acid flux on the Na+ gradient. This reinterpretation agrees with the hypothesis of VIDAVER[7] who suggested that the Na+ gradient is coupled to and energetically drives amino acid uptake in the pigeon red blood cell.
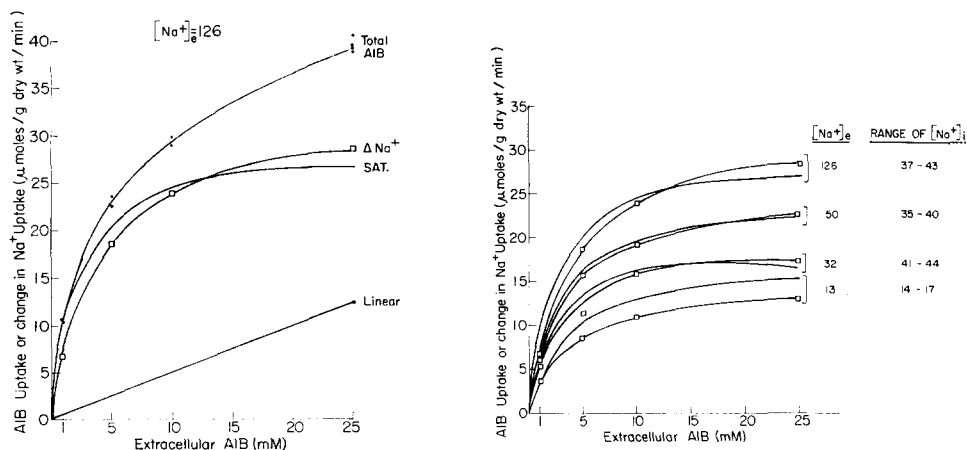

Fig. 1. Correlation of change in Na+ uptake with α-aminoisobutyric acid (AIB) uptake for 1-min incubations at 37°. Uptakes were measured for initial extracellular α-aminoisobutyric acid concentrations of 1, 5, 10 and 25 mM. Change in Na+ uptake (the curve designated by □ and △Na+) is the difference between Na+ uptake in the presence of α-aminoisobutyric acid and the uptake in the absence of α-aminoisobutyric acid. The curve labeled SAT. is the saturatable component of α-aminoisobutyric acid uptake calculated by subtracting the linear component from the total α-aminoisobutyric acid uptake.

Fig. 2. Correlation of change in Na+ uptake with the saturatable component of α-aminoisobutyric acid (AIB) uptake at different extracellular sodium concentrations for 1-min incubations at 37°. Uptakes were measured with initial extracellular α-aminoisobutyric acid concentrations of 1, 5, 10 and 25 mM. The change in Na+ uptake (□—□) and the saturatable α-aminoisobutyric acid uptake (plots with no associated symbol) were calculated as described for Fig. 1. The extracellular and intracellular concentrations of Na+ are in mequiv/l of extracellular and mequiv/kg of intracellular water.

The critical question now is whether Na^+ actually enters the cell along with the amino acid or whether it merely participates in some secondary reaction accompanying transport. This same problem has been the focus of recent research on transport in the intestine. CSAKY[8] has shown that sodium is necessary for active intestinal transport of amino acids, and SCHULTZ AND ZALUSKY[9] have shown that the flux of Na^+ from mucosal to serosal surface and the short-circuit current increase when alanine is transported and suggest a 1:1 stoichiometry between the two on the basis of their finding of a Michaelis–Menten type of relation between the increase in short-circuit current and the alanine concentration on the mucosal side. They further suggest that a ternary complex, Na^+–amino acid –carrier is involved in the intestinal transport of amino acids, and that the transport is driven by the Na^+ gradient. The same hypothesis had been proposed by CRANE, MILLER AND BIHLER[10] for sugar transport in the intestine.

We have attacked this problem directly by measuring the simultaneous uptakes of ^3H-labeled α-aminoisobutyric acid and of $^{22}Na^+$ for incubation times of 1 min at 37° for a series of different extracellular concentrations of Na^+ and α-aminoisobutyric acid. The methods and procedures used have been described previously[11,12]. For this study the Ehrlich ascites cells were not subjected to osmotic shock. We have found a 1:1 relationship between the increase in Na^+ uptake and the saturatable component of α-aminoisobutyric acid uptake. Fig. 1 shows that when the linear component of the α-aminoisobutyric acid uptake (referred to as the Na^+-insensitive component by INUI AND CHRISTENSEN[13]) is subtracted the remaining, saturatable and Na^+-sensitive, uptake of α-aminoisobutyric acid is approximately the same as the increase in Na^+ uptake. As is shown in Fig. 2, this 1:1 relationship holds over a wide range of extracellular concentrations of Na^+ and α-aminoisobutyric acid, for both inwardly and outwardly directed Na^+ gradients. The intracellular and extracellular Na^+ levels are given in Fig. 2 for each set of curves. In the experiment in which the extracellular Na^+ was 32 mM and intracellular Na^+ was in the range 41–44 mM the α-aminoisobutyric acid uptake in 1 min was not concentrative at the higher levels of α-aminoisobutyric acid but at 1 mM α-aminoisobutyric acid the intracellular concentration reached was 2 mM. Thus these experiments provide direct evidence for a 1:1 stoichiometry between α-aminoisobutyric acid and Na^+ uptake as well as evidence against the hypothesis that the Na^+ gradient is the sole source of the energy for the transport of α-aminoisobutyric acid.

We have run similar experiments with L-phenylalanine which is taken up almost entirely by the Na^+-insensitive L system and have found no increase in uptake of Na^+ accompanying uptake of L-phenylalanine.

We interpret our results as indicating that one Na^+ is bound to the carrier of the Na^+-sensitive A system but that no Na^+ is bound to the carrier of the Na^+-insensitive L system. It is unlikely that the Na^+ is moving independently as a counter-ion of α-aminoisobutyric acid because both α-aminoisobutyric acid and phenylalanine are neutral amino acids and one would then expect it to move with phenylalanine as well as with α-aminoisobutyric acid. Hence if it is moving as a counter-ion it must be as a counter-ion for the carrier for α-aminoisobutyric acid. Although our experiments do not exclude this possibility it seems to us that by far the most likely explanation of the 1:1 stoichiometry and the Michaelis–Menten type of relationship between the α-aminoisobutyric acid flux and the extracellular concentration of

sodium[13] is that the sodium and the α-aminoisobutyric acid are bound to the same carrier.

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An electron spin resonance signal in brain microsomes

Microsomal preparations from liver and adrenal cortex have been examined by ESR spectroscopy[1-5]. Studies on brain microsomes with this technique have not been reported.

Active cation transport and ATPase activity have been studied extensively, in brain as well as in other tissues[6]. A connection between microsomal electron transport and the active transpoit of ions has been suggested (*e.g.* refs. 6-8), but experimental evidence has been lacking. Previous work[9,10] using frog skin suggested that unpaired electrons participate in active cation transport. We report here preliminary findings showing that a free radical is in some way connected with microsomal ATPase.

Rats were killed by decapitation after light ether anesthesia. (One preparation from rats that received no ether was also examined; we observed no difference in the signal.) The brains were quickly removed and chilled. The microsomal pellet was

Abbreviation: ESR, electron spin resonance.