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THE EFFECT OF EXTRACELLULAR SODIUM CONCENTRATION ON THE KINETICS OF α-AMINOISOBUTYRIC ACID TRANSPORT IN THE RAT KIDNEY CORTEX SLICE

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SUMMARY

The kinetics of α-aminoisobutyric acid (AIB) accumulation by the rat kidney cortex slice under conditions of varying extracellular sodium concentration were investigated. Reduced extracellular sodium concentration resulted in a diminished initial uptake of amino acid and reduced influx under steady-state conditions. Efflux was accelerated under steady-state conditions and initial efflux was accelerated by reducing extracellular sodium concentration to below intracellular concentration. These findings are consistent with the concept of a linked sodium and amino acid transport mechanism.

INTRODUCTION

Previous investigations from this laboratory have demonstrated that, in the rat kidney cortex slice, transport of the neutral amino acid α-aminoisobutyric acid (AIB) is directly dependent upon extracellular sodium ion concentration over a wide range of sodium concentrations[1]. Cationic molecules such as tris(hydroxymethyl)aminomethane (Tris), lithium, lysine, and potassium failed to substitute effectively for sodium. Although an increase or decrease from physiological potassium concentrations inhibited amino acid transport, the primary importance of the sodium ion concentration was established.

Evidence has been presented suggesting that the transmembrane movement of sodium and non-electrolytes is intimately linked[2,3]. Other data has been presented,

Abbreviation: AIB, α-aminoisobutyric acid.

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however, supporting the possibility of a requirement of a non-specific energy source for intracellular sodium[4]. The present study, undertaken to define the kinetics of AIB transport under conditions of varying extracellular sodium concentration, indicates that lowering the sodium concentration decreases the initial and steady-state influx of the amino acid. When the extracellular sodium concentration is lower than the intracellular concentration, the initial and steady-state efflux of AIB is increased. These data support the concept of a linked sodium–amino acid transport process in the rat kidney cortex slice.

METHODS

Male Sprague–Dawley rats weighing 120–160 g, used in all experiments, were fed a Purina (R) chow diet and water *ad libitum* until being sacrificed by stunning and decapitation. The techniques employed for the preparation of kidney cortex slices, aerobic incubation in Krebs–Ringer bicarbonate buffer (pH 7.4) at 37°, estimation of total tissue water, determination of extracellular space with [^{14}C]inulin, calculation of intracellular and medium concentrations of ^{14}C-labeled amino acids, the application of Michaelis–Menten kinetics to amino acid transport studies, and the techniques for steady-state incubation have been described in detail previously[5,6]. Multicompartmental analysis of steady-state kinetics was performed as previously described by ROSENBERG, BERMAN AND SEGAL[6].

Media with varying sodium concentrations were prepared by substituting Tris in an isomolar fashion for sodium. Tris was chosen because it was cationic, acted as a buffer, and at physiological pH, where it is approx. 80% ionized, served adequately to maintain osmolarity[1].

Efflux was measured by incubating tissue in Krebs–Ringer bicarbonate buffer (pH 7.4) at 37° containing 0.065 mM [^{14}C]AIB for 60 min after which time the tissue was rinsed and transferred to Krebs–Ringer bicarbonate buffer, 72 mequiv/l sodium buffer, or 0 mequiv/l sodium buffer all without AIB. Aliquots of the AIB-free buffers were taken at 4-min intervals following transfer. After the fifth aliquot the tissue was removed, rinsed, blotted, weighed, and placed in distilled water. The tissue amino acid pool was equilibrated with the water by boiling for 6 min. Aliquots of the media and of the aqueous tissue extract were counted in a liquid-scintillation spectrometer as previously described[5]. Tissue radioactivity at the time of transfer to AIB-free medium was calculated by adding the total of all radioactivity appearing in the medium to the radioactivity remaining in the tissue at the completion of the study. Using this value as 100%, the per cent of radioactivity remaining in the tissue at each time point after transfer to AIB-free medium was calculated and plotted on semi-log paper. From the curves thus derived an efflux constant could be calculated.

MATERIALS

[1-^{14}C]AIB, specific activity 3.39 mC/mmole, was purchased from Isotope Specialty Company. Tris (hydroxymethyl)aminomethane was purchased from the Sigma Chemical Company.

Prior to use, AIB was shown to be chromatographically pure using a one-dimensional descending paper system in butanol–acetic acid–water (4:1:2, by vol.).

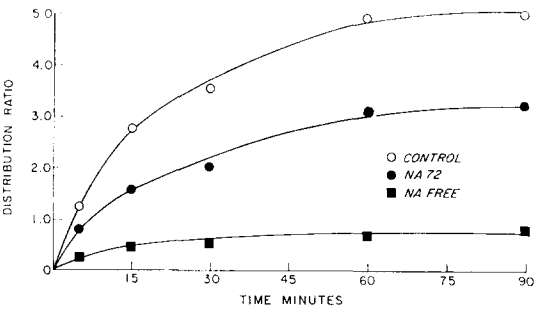

Fig. 1. Plot of the distribution ratios for AIB *vs.* time under conditions of varying extracellular sodium concentration. Tissues were incubated for 60 min with 0.065 mM AIB and then transferred to flasks with 0.065 mM [^{14}C]AIB. For each set of tissues the pre- and post-transfer media differed only in the presence of labeled AIB in the latter. Steady-state conditions for AIB uptake by the kidney cortex slices are reached by 60 min. Time 0 on the figure represents the time of transfer to flasks with [^{14}C]AIB and therefore the curves represent steady-state accumulation. The calculated data of Table I are obtained from these curves. Each point represents the average of 6 determinations. The steady-state distribution ratios in sodium-free buffer are not significantly different from 1.0.

After incubation chromatography of the aqueous tissue extract revealed that over 90% of the radioactivity was recovered at the appropriate R_F for AIB.

RESULTS

Steady-state kinetics

The curves of the uptake of [^{14}C]AIB added under steady-state conditions to media of varying sodium content are shown in Fig. 1. Fractional turnover rates of

TABLE I

EFFECT OF ALTERATION OF EXTRACELLULAR SODIUM ON KINETICS OF AIB TRANSPORT IN RAT KIDNEY CORTEX SLICES

Incubation of 3 rat kidney cortex slices (60–100 mg) was carried out in 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4), containing 0.065 mM unlabeled AIB. After 60 min the tissues were removed, blotted, transferred to 2 ml of buffer with 0.065 mM [^{14}C]AIB, and incubated for varying times shown in Fig. 1.

Medium*	λMI ⇆ λIM	Intracellular space
Experimental condition	*Fractional turnover rate** (min^{-1})*	
	λIM	λMI
Control	0.0088 ± 0.0009	0.074 ± 0.011
Sodium 72 mequiv/l	0.0043 ± 0.0005	0.057 ± 0.009***
Sodium 0 mequiv/l	0.0027 ± 0.0004	0.153 ± 0.024

* Site of initial activity.

** Expressed as turnover rate ± S.D.: λIM, rate constant for movement into intracellular space from medium; λMI, rate constant for movement into medium from intracellular space.

*** Not significantly different from control.

AIB calculated by a multicompartmental analysis of these data are presented in Table I. A reduction in medium sodium concentration from 140 to 72 mequiv/l resulted in a 51% reduction in influx but no significant change in efflux. A further reduction in medium sodium concentration to below intracellular concentration (*i.e.*, medium sodium 0 mequiv/l) resulted in a further decrease in influx (69% reduction) and, in addition, in a doubling of efflux.

Influx

An evaluation of the effect of sodium concentration on the linear uptake phase of AIB accumulation was undertaken. Data from these studies are plotted according to the double-reciprocal method of LINEWEAVER AND BURK[7] (Fig. 2). In an analysis of these data it was assumed that a portion of biological transport behaved as a saturable system and a correction was made for that portion of accumulation due to physical diffusion[5]. The results indicate that reducing extracellular sodium concentration reduces the apparent affinity (*i.e.*, increases the K_m of AIB for its transport mechanism, but does not alter the maximum rate (v_{max}) at which the amino acid is accumulated.

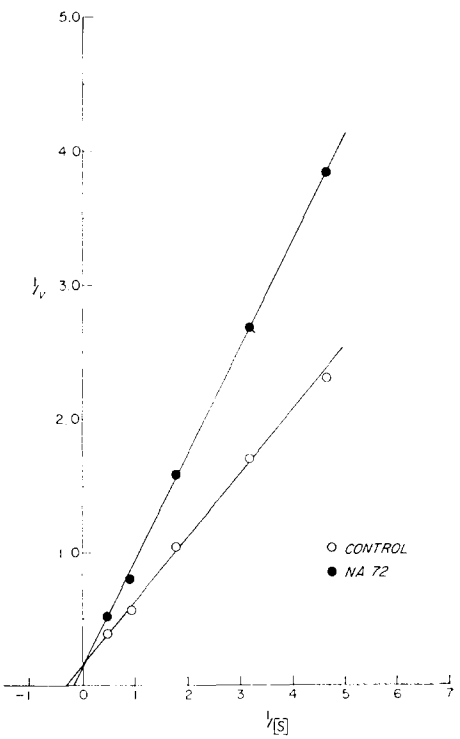

Fig. 2. LINEWEAVER–BURK plot of the effect of increasing external AIB concentration [S] on the saturable transfer (v) in the presence of control and reduced (72 mequiv/l) concentration of extracellular sodium. The duration of incubation was 30 min and each data point represents the average of 6 determinations. The reciprocals of the external concentration [S] expressed in mM are plotted against the reciprocal of the observed v values expressed as mM per 30 min.

Efflux

Studies of the linear phase of efflux (Fig. 3) indicated that lowering medium sodium from 140 to 72 mequiv/l was without effect on the efflux of AIB from kidney cortex slices. However, further reduction of medium sodium to below intracellular concentrations (medium sodium 0 mequiv/l) resulted in a significant acceleration of efflux.

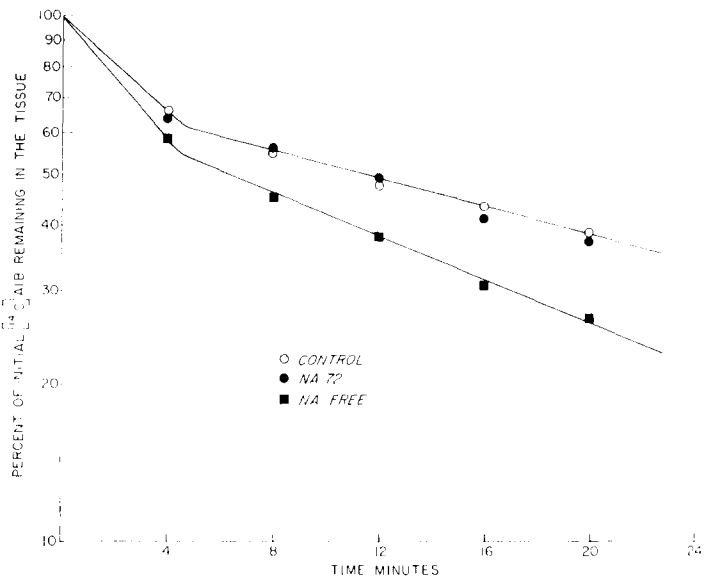

Fig. 3. The effect of reducing extracellular sodium concentration on the efflux of [^{14}C]AIB from rat kidney cortex slices. All slices were incubated in Krebs–Ringer bicarbonate buffer with 0.065 mM [^{14}C]AIB. After 60 min slices were transferred into AIB-free buffers containing 0 mequiv/l, 72 mequiv/l or control amounts of sodium. The media were sampled every 4 min and the amount of radioactivity remaining in the tissue was calculated for each time point. The per cent of initial [^{14}C]AIB remaining in the tissue is plotted against time on a semi-log graph. Each point represents the average of 4 or more determinations.

DISCUSSION

The role of sodium ion in the transmembrane transport of non-electrolytes has been the subject of controversy for some time. CSAKY[4] observed that numerous chemically unrelated non-electrolytes were actively transported by intestine only in the presence of sodium and suggested that the requirement for sodium was non-specific. He further noted that of a series of molecules substituting for sodium, those which best penetrated the cell most inhibited transport. This latter observation, coupled with his apparent demonstration that carrier-linked non-active transport did not require sodium, led CSAKY to postulate that sodium was required intracellularly for the production of energy which could be used non-specifically. CRANE[2] has proposed an alternative hypothesis which states that sodium is required extracellularly. The transmembrane transport of sodium and non-electrolytes is viewed as a linked process, the non-electrolyte entering the cell along a sodium gradient and energy being required to extrude sodium. By reducing extracellular sodium concentration,

CRANE and others[2,8] have demonstrated a decrease in the apparent affinity of non-electrolytes for their transport mechanisms. CRANE's studies of sugar transport in the intestine have been supported by studies of amino acid transport in gut[3,9] and in diaphragm[8]. Most recently, FUISZ, SCHULTZ AND CURRAN[9] have conclusively demonstrated the importance of sodium in an extracellular position for alanine transport in gut.

The present study indicated that the influx of AIB into rat kidney cortex slices diminishes as extracellular sodium concentration is reduced. When the linear phase of amino acid accumulation is studied in order to approximate initial rates and the data obtained are analyzed, assuming uptake obeys saturation kinetics, it is noted that reducing extracellular sodium reduces the apparent affinity of AIB for a transport site, but does not affect the maximal rate of uptake.

To determine whether this effect occurred only during the initial uptake phase of accumulation, studies were repeated under steady-state conditions. Again, influx was inhibited by removing extracellular sodium. However, when extracellular sodium was reduced to 0 mequiv/l, efflux appeared to be accelerated.

To determine whether the acceleration of efflux was the result of prolonged exposure to low sodium, tissue was preloaded with amino acid in Krebs–Ringer bicarbonate buffer and transferred to amino acid-free low-sodium buffer where the linear phase of efflux could be studied. Again, a stimulation of efflux was noted when extracellular sodium was reduced to 0 mequiv/l. Extracellular sodium concentration of 72 mequiv/l did not affect the rate of efflux. Earlier studies[1] demonstrated that exposure to sodium-free buffer for up to 60 min did not irreversibly damage the tissue.

Our data are most consistent with the hypothesis of CRANE and support the concept that the rate of influx is dependent upon the extracellular sodium concentration. That amino acid and sodium are intimately linked in transmembrane transport is suggested by the altered K_m for AIB transport with decreased extracellular sodium. The data obtained from efflux measurements further suggest that the transmembrane sodium gradient plays an important role in amino acid transport and that amino acid movement is directly related to the sodium gradient regardless of its direction.

These interpretations of the role of sodium in amino acid transport apply only to the neutral amino acids totally dependent upon the presence of sodium. It is obvious that other mechanisms must be operating to explain the sodium-independent transport of the dibasic amino acids and histidine[1].

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