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THE EFFECT OF EXTRACELLULAR SODIUM CONCENTRATION ON α-METHYL-D-GLUCOSIDE TRANSPORT BY RAT KIDNEY CORTEX SLICES

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SUMMARY

The kinetics of α-methyl-D-glucoside accumulation by rat kidney cortex slices under conditions of varying extracellular sodium concentration were investigated. Extracellular sodium reduction below 144 mequiv/l resulted in a diminished initial uptake and reduced influx calculated by steady-state analysis of a two-compartment system. At 72 mequiv/l extracellular sodium efflux was decreased to the same extent as influx resulting in the same steady state concentration that was observed at 144 mequiv/l sodium. At 36 mequiv/l the steady state concentration was below that observed at 144 mequiv/l sodium because of a disproportionate decrease of influx. In the complete absence of extracellular sodium, no concentration gradient was achieved and efflux had become increased. Low extracellular sodium was associated with an increase in the apparent K_m of transport without affecting the V. The apparent K_i for sodium appeared to be in the 40 to 59 mequiv/l range. The presence of 20 mM α-methyl-D-glucose prompted the accelerated efflux of the sugar from the tubule cells in a normal fashion despite a low extracellular sodium concentration.

INTRODUCTION

A relationship of organic solute transport to Na^+ in a variety of tissues has been well established [1]. Extensive data on the transport interaction of sugars with Na^+ in intestinal mucosa and cell preparations have led to theoretical concepts of membrane function [2]. Relatively meager knowledge, however, has been attained regarding the effects of Na^+ on sugar transport in kidney cortex.

Sodium-dependent transport of glucose has been observed in perfused rat [3] and frog kidney [4,5] and in rabbit kidney cortex slices [6]. Kleinzeller and associates [6–8] have demonstrated sodium-dependent uptake of other hexoses including α-methyl-D-glucoside, but in addition, a sodium-independent uptake of α-deoxyhexoses by rabbit kidney cortex. Data from this laboratory have indicated effects of sodium deprivation on α-methyl-D-glucoside transport by human kidney cortex [9] and on galactose [10] and α-methyl-D-glucoside [11] uptake by rat kidney cortex slices. Our previous investigation has demonstrated that the uptake

of non-metabolizable α-methyl-D-glucoside by rat kidney cortex is directly dependent upon extracellular Na^+ concentration from 0 to 72 mequiv/l [11]. The present study was undertaken to define the kinetics of α-methyl-D-glucoside transport under conditions of varying extracellular sodium concentration. Our results form the basis of this report.

EXPERIMENTAL

Male Sprague–Dawley rats weighing 150–200 g used in all experiments were fed a Purina Chow diet and water ad libitum until being sacrificed by decapitation. The techniques employed for the preparation of kidney cortex slices, aerobic incubation in Krebs–Ringer bicarbonate buffer (pH 7.45) at 37 °C, calculation of intracellular and medium concentration of α-methyl-D-[U-^{14}C]glucoside concentration and the application of Michaelis–Menten kinetics to sugar transport studies has been described in detail previously [9,11]. Conditions for producing Na^+ depletion by Tris substitution [12] have been published.

Results are expressed as the distribution ratios, the cpm/ml of intracellular fluid to cpm/ml medium. The distribution ratio represents a concentration gradient since α-methyl-D-glucoside is not metabolized in the tissue [11]. Concentration of the sugar in the tissue is obtained by multiplying the distribution ratio by the medium substrate concentration. This was done to derive the velocity in concentration dependence experiments.

The extracellular space and total tissue water were 25% and 80% of wet tissue weight in Krebs–Ringer buffer [13]. Values for these parameters in sodium-depleted buffer have been used in the calculations [12].

Efflux was measured as described before [11] by incubating slices with α-methyl-D-[U-^{14}C]glucoside for 60 min after which the tissue was removed, quickly rinsed in physiological saline, blotted and transferred to flasks containing 3 ml of media. The vessels were then gassed and sealed. At 3-min intervals the flasks were opened and the medium sampled for radioactivity. At the end of 18 min the tissues were removed and the ^{14}C content of the tissue assessed. The total counts effluxed into the medium and the counts remaining in the tissue after 18 min were summed to determine the label present at the onset of the efflux phase.

The two compartment analysis of α-methyl-D-glucose kinetics was performed as described by McNamara et al. [14] based on the multicompartmental analysis of steady state kinetics described for kidney slices by Rosenberg et al. [15]. All linear curves were fitted to the data with a Monroe Computer (Model 1775).

α-Methyl-D-[U-^{14}C]glucoside (73.4 Ci/mole) was obtained from Calbiochem, Los Angeles, Calif. This was found to give one radioactive spot corresponding to a known standard on thin layer chromatography [11]. Unlabeled α-methyl-D-glucoside was purchased from the Pfanstiehl Co., Waukegan, Ill. and found to be pure and free of glucose by gas–liquid chromatography [11].

RESULTS

Effect of Na^+ on concentration uptake

The uptake of α-methyl-D-glucoside in Krebs–Ringer bicarbonate buffer (Na^+, 144 mequiv/l) and Tris buffers containing 72, 36 and 0 mequiv/l of Na^+

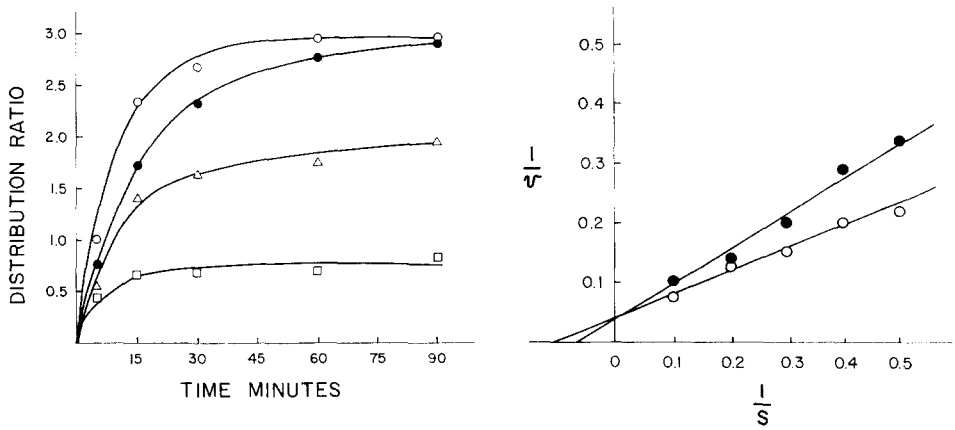

Fig. 1. The uptake of α-methyl-D-glucoside by rat kidney cortex slices incubated in buffers of various sodium concentrations. Three slices, one from each of three animals, total weight 20–45 mg were incubated in 2 ml of buffer, pH 7.4, containing 2 mM sugar and 0.2 μCi/ml of label at 37 °C in a Dubnoff shaker. Uptake is designated by the distribution ratio, the ratio of cpm/ml intracellular fluid to cpm/ml medium. Each point is an average of three determinations. Sodium concentration in mequiv/l: ○—○, 144; ●—●, 72; △—△, 36; □—□, 0.

Fig. 2. Lineweaver–Burk plot of the effect of lowering the medium sodium concentration on α-methyl-D-glucoside uptake. v is velocity expressed as mmoles/l per 30 min and has been corrected for diffusion. S is α-methyl-D-glucoside concentration in mM. Each point is an average of triplicate determinations. ○—○, 144 mequiv/l; ●—●, 50 mequiv/l sodium.

is shown in Fig. 1. This range of Na^+ concentration was selected since very little difference in sugar uptake had been observed previously between 108 and 170 mequiv/l [11]. The data in Fig. 1 reveal that lowering the Na^+ consistently diminishes the distribution ratio observed at 5 min of incubation. At 72 mequiv/l of Na^+, the concentration gradient of α-methyl-D-glucoside, however, reaches the same steady state value as the control (144 mequiv/l). Further reduction of the medium Na^+ level to 36 mequiv/l produces a steady state concentration gradient lower than that seen at 72 or 144 mequiv/l. With total removal of sodium from the medium the steady state distribution ratio becomes 0.75.

The effect of reducing the medium Na^+ to 50 mequiv/l on the velocity of uptake was studied at various α-methyl-D-glucoside concentrations and Fig. 2 is a Lineweaver–Burk double reciprocal plot of the data points obtained. Reducing the sodium concentration did not affect the maximum velocity but raised the apparent K_m from 9 to 16.6 mM. Calculation of an apparent K_i from these values for sodium gives a value of 59 mequiv/l. Fig. 3 is a Dixon plot [16] of the reciprocal of the uptake velocity of 2, 5 and 10 mM α-methyl-D-glucoside at 30 min of incubation versus the sodium concentration. This would be analogous to considering the diminishing sodium concentration as an inhibitor. The intersection of the lines above the abscissa is considered evidence that low sodium decreases the velocity of uptake by affecting the saturable binding site for sugar transport. A perpendicular dropped from the intersection of the lines should give an apparent K_i. Since these curves do not intersect in a single point an approximate value can only be estimated ranging from 40 to 58 mequiv/l.

Kinetic parameters affected by sodium deprivation

In previous studied uptake curves obtained by incubating with 2 mM labeled sugar from the outset are essentially the same as those obtained by incubating with unlabeled compound to a steady state before the addition of radioactive tracer α-methyl-D-glucoside [14]. Therefore, assuming a closed two compartment system we have analyzed the data represented in Fig. 1. The solution of the parameters of the two-compartment system is shown in Table I. When medium sodium is

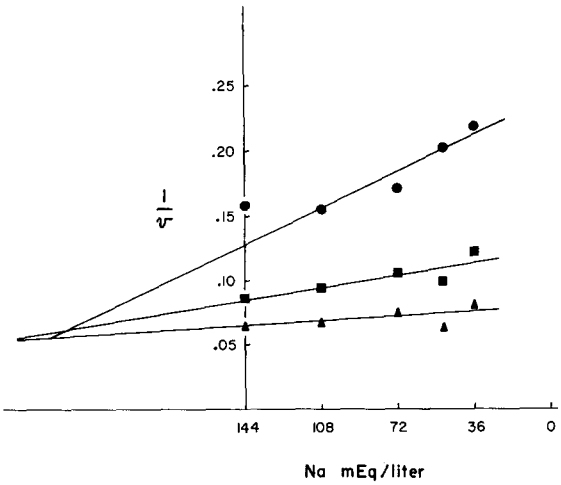

Fig. 3. Dixon plot of the relationship of velocity of α-methyl-D-glucoside uptake at various concentrations to medium sodium. Each point is an average of triplicate incubation flasks. v is velocity expressed as mmoles/l per 30 min. Sugar concentrations were: ●—●, 2 mM; ■—■, 5 mM; ▲—▲, 10 mM.

TABLE I

INFLUENCE OF EXTRACELLULAR SODIUM ON KINETICS OF α-METHYL-D-GLUCOSIDE TRANSPORT

Medium	λ_{IM} ⇌ λ_{MI}	Intracellular space				
Na^+ concentration (mequiv/l)	Medium pool size (μmoles)	Steady-state distribution ratio	Steady-state intracellular pool (μmole)*	Fractional turnover rate (min^{-1})**		Net flux (μmole/min per 100 mg final wet wt)
				λ_{IM}	λ_{MI}	
144	4	3.0	0.330	0.00660	0.0800	0.02640
72	4	3.0	0.330	0.00418	0.0507	0.01672
36	4	1.8	0.198	0.00330	0.0667	0.01320
0	4	0.75	0.083	0.00220	0.1067	0.00880

* All calculations are based on 100 mg of tissue and an intracellular space of 55% of wet tissue weight.

** The rate constants are related by the equation: $M \cdot \lambda_{IM} = (\text{intracellular space}) \cdot \lambda_{MI}$ where M is the medium concentration.

decreased from 144 to 72 mequiv/l there is no change in the steady state pool size despite a reduction in the net flux. This occurs with the same 36% reduction in both the fractional rate constants for influx and efflux. The further reduction of Na^+ to 36 mequiv/l caused a further reduction in the λ_{IM} to 0.0033 min^{-1} for entry but no concomitant further reduction in λ_{MI}. The imbalance in the percent change of the fractional turnover rates at 36 mequiv/l is accompanied by a decrease in the steady state distribution ratio and intracellular pool. In the complete absence of sodium, λ_{IM} is one-third of the value observed at 144 mequiv/l while λ_{MI} appears to have become greater than that at 144 mequiv/l (0.1067 vs 0.080 min^{-1}). With these changes there is a further decrease in the intracellular pool and the steady state distribution ratio is 0.75.

A plot of λ_{IM} vs medium sodium concentration reveals a linear relationship with a decrease of 0.00003 min^{-1}/mequiv as Na^+ is decreased from 144 to 0 mequiv/l. There is no linear relation between λ_{MI} and Na^+ concentration with λ_{MI} decreasing as Na^+ is lowered but increasing above the value at 144 mequiv/l as sodium is totally depleted.

Observed changes in efflux with low sodium

Direct observations of efflux were made from slices incubated in buffer with 144 and 50 mequiv/l Na^+. The results are shown in Fig. 4 and indicate that α-methyl-D-glucoside effluxes more slowly when the Na^+ concentration is reduced. The rate constant for efflux at 144 mequiv/l is 0.087 min^{-1}. In slices where the sugar was taken up from media containing 50 mequiv/l the rate constant was decreased to 0.055 min^{-1}. This 36% reduction in the rate constant corresponds to the changes in the fractional turnover rates for efflux calculated from the uptake curves and shown in Table I.

Fig. 4 also shows that even if the uptake of sugar takes place in buffer with 144 mequiv/l Na^+, the subsequent transfer of the slices to medium with 50 mequiv/l results in a slowing of efflux after 6 min with a resulting diminution of the rate constant to 0.062 min^{-1}.

Accelerated efflux

Previous experiments [17] have shown that extracellular unlabeled α-methyl-D-glucoside will accelerate the loss of labeled compound from the slice. Such results have been interpreted as evidence for a mobile carrier system [18,19]. In order to observe the effect of sodium deprivation on such a system the studies shown in Fig. 5 have been performed. A low concentration of the sugar (0.07 mM) was incubated with cortical slices in 144 mequiv/l Na^+ buffer for 60 min after which the slices were placed either in 50 mequiv/l Na^+ alone or in 50 mequiv/l buffer containing 20 mM α-methyl-D-glucoside. Fig. 5A shows that though the media was low in Na^+ and the efflux of α-methyl-D-glucoside slowed (K=0.069 min^{-1} compared to 0.087 in Na^+ 144 mequiv/l, Fig. 4) the large amount of extracellular sugar accelerated the efflux K=0.103 min^{-1}.

Fig. 5B shows a similar experiment in which the uptake and efflux phases were both carried out in buffer containing 50 mequiv/l Na^+. Efflux into buffer alone occurred with a rate constant of 0.052 min^{-1}. The efflux K was 0.066 min^{-1} in the same buffer containing 20 mM α-methyl-D-glucoside.

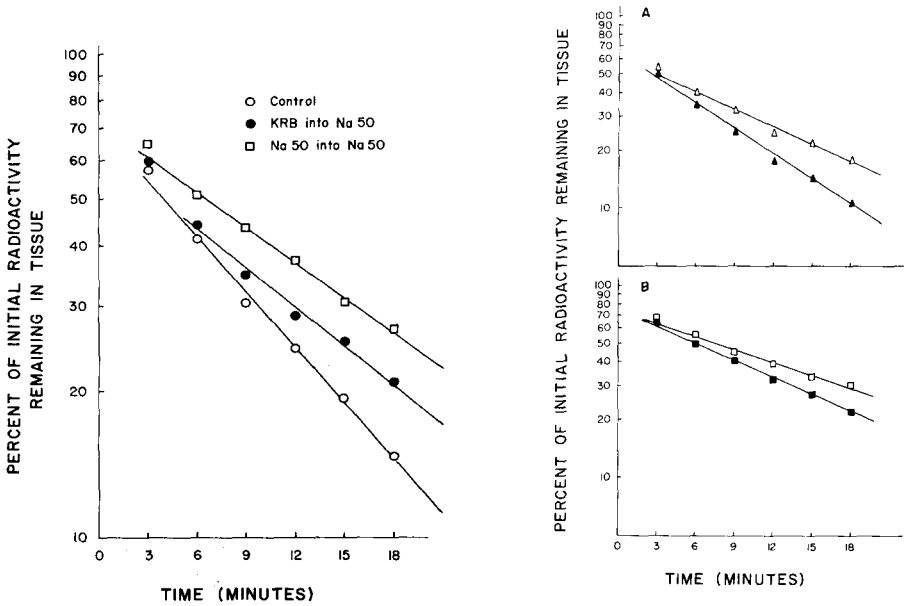

Fig. 4. Effect of low medium sodium on the efflux of α-methyl-D-glucoside from rat kidney cortex slices. Slices accumulated the radioactive glucoside for 60 min at 37 °C from medium containing 2 mM and 0.2 μCi/ml of label. ○—○, uptake and efflux medium containing 144 mequiv/l sodium; ●—●, uptake in medium containing 144 mequiv/l sodium and efflux into medium containing 50 mequiv/l sodium; □—□, uptake and efflux from medium with 50 mequiv/l sodium. Each point is the average of duplicate determinations.

Fig. 5. Effect of low-medium sodium on efflux into medium containing 20 mM α-methyl-D-glucoside. Slices accumulated α-methyl-D-[U-^{14}C]glucoside, 0.07 mM, for 60 min at 37 °C. In A uptake occurred in medium containing 144 mequiv/l sodium and efflux of glucoside was followed in medium with 50 mequiv/l sodium. △—△, efflux into buffer alone; ▲—▲, efflux into buffer with 20 mM α-methyl-D-glucoside. In B uptake and efflux both took place in buffer with 50 mequiv/l sodium. □—□, efflux into buffer alone. ■—■, efflux into buffer with 20 mM glucoside.

DISCUSSION

The massive data on the role of Na^+ in sugar transport in the intestine has been recently reviewed and put into perspective by Kimmich [2]. The exact mechanism is still unknown but the tendency seems to be against the postulate of Csaky [20] that sodium is required for intracellular production of energy which can be used non-specifically. Crane's [21] alternative that sodium is required extracellularly, the transport of non-electrolyte and sodium being viewed as a linked process, is favored. Crane and others (see reviews by Crane [21] and Schultz and Curran [1]) have demonstrated a decrease in the apparent affinity of non-electrolytes for their transport mechanisms in intestine by reducing extracellular sodium concentration. Our own data in rat and human kidney cortex slices that decreased extracellular sodium alters the apparent transport K_m for sugar entry are consistent with the Crane hypothesis.

It should be pointed out that there may be species differences in the effects

of lower sodium for in rabbit kidney slices it is the V of entry that is altered and not the K_m [7,8]. Indeed, at physiological concentrations, the uptake of D-glucose by membrane vesicles [22] and "binding" to an isolated brush border preparation of rabbit kidney tubules [23] has been reported to be independent of sodium. With isolated rat kidney brush border membranes Frasch et al. [24] showed that glucose binding to the phlorizin receptor site is sodium dependent.

Our data in Figs 2 and 3 indicating that the level of external Na^+ affects the binding of α-methyl-D-glucoside to the transport site do not distinguish whether Na^+ is a ligand for the site or whether Na^+ affects the site allosterically as proposed by Crane [21]. The apparent K_i determined to be 40–58 mequiv/l Na^+ may be analogous to the K_m of sodium for its binding site. Thus, in this concentration range the velocity of entry would be half maximal with maximum entry velocity calculated to be 80 to 116 mequiv/l Na^+. This is consistent with our observation that a maximum velocity of entry occurs at Na^+ levels of 108 mequiv/l [11].

A decrease in extracellular sodium also results in a decrease in efflux but the total absence of sodium reverses this. Most of the reported observations on sodium effects on non-electrolyte transport have dealt with the influx process [1]. Indeed, the Crane hypothesis speculates only on the relationship of extracellular sodium to influx. The nature of the "trans" effect of external sodium on the transport mechanism for sugar on the inner aspect of the cell membrane remains to be determined. Changes in sodium outside the cell may be reflected by changes of intracellular electrolytes or other changes in the intracellular environment.

Kinetically, lowering of extracellular sodium decreased the entry rate constant but because of the concomitant decrease in exit rate constant there is no change in the steady state concentration gradient until the concentration of sodium is below 72 mequiv/l. These results are different from those observed with α-amino-isobutyric acid [25]. At 72 mequiv/l Na^+ the amino acid influx was slowed but efflux was unchanged resulting in a decrease in the steady concentration gradient. At 0 mequiv/l Na^+ the kinetics of sugar and amino acid entry and exit were similar with marked slowing of entry and an increase in exit rate constants.

Although the kinetic analysis of α-methyl-D-glucoside uptake by rat kidney cortex slices is satisfied by a simple two-compartment analysis where influx and efflux may be calculated, the actual localization of sugar entry and exit to luminal or anti-luminal cell borders cannot be determined. As pointed out by Schultz et al. [26] for the intestine there are at least four separate undirectional fluxes which contribute to the net solute flux. Deetjen and Boylan [7], in this regard, have demonstrated no actual glucose movement from peritubular capillary to tubule lumen in microperfusion studies. The asymmetry of the kidney cortex cell with regard to Na^+–K^+-dependent ATPase and the sodium pump mechanism has been shown by the localization of this enzyme to the basal membrane and its complete absence from luminal brush border [28,29].

In terms of the carrier concept [18,19] our data showing accelerated efflux in the presence of low external sodium suggest that a moderate decrease in affinity of the transport binding site for sugar does not alter the difference in mobility of free and loaded carrier responsible for the phenomenon.

The interpretations of the role of sodium in sugar transport in rat kidney cortical cells should not be generalized from our observations on α-methyl-D-

glucoside. Its transport has many characteristics of the glucose–galactose system but there are differences [11]. Indeed, galactose transport in both rabbit [8] and rat [10] has a component which is non-sodium dependent. In addition, Kleinzeller et al. [7] have pointed out that in rabbit cortex cells 2-deoxyhexose transport is not sodium dependent. It is obvious that other mechanisms may be operative to explain the sodium-independent transport of these sugars.

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