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Effect of Temperature on Dicarboxylate Transport in Plasma Membrane Vesicles of Rabbit Proximal Tubule

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=ABSTRACT=

The temperature dependence of Na^+-dependent succinate uptake was studied in brush border (BBMV) and basolateral (BLMV) membrane vesicles isolated from the rabbit kidney cortex. The succinate uptake was markedly altered by temperature in a similar fashion in both membranes. The temperature dependence was characterized by a nonlinear Arrhenius plot with a break point at 22 and 25℃ for BBMV and BLMV, respectively. The activation energy was 3.91 and 17.09 kcal/mole at above and below the break point, respectively, for BBMV; 2.65 and 14.05 kcal/mole, respectively, for BLMV. When temperature increased from 20 to 35℃, the Vmax of succinate transport increased from 3.49 ± 0.11 to 5.90 ± 0.86 nmole/mg/5 sec for BBMV and from 2.86 ± 0.25 to 3.63 ± 0.32 nmole/mg/5 sec for BLMV, with no change in Km in both membranes.

These results suggest that renal dicarboxylate transport is similarly sensitive to a change in membrane physical state in BBMV and BLMV,

Key Words: Dicarboxylate transport, Temperature, Membrane vesicles, Proximal tubule

INTRODUCTION

Krebs cycle intermediates, such as succinate, α-ketoglutarate, and citrate, are actively taken up by renal proximal tubular cells from the luminal (Sheridan et al., 1983) as well as from the peritubular side (Cohen & Wittmann, 1963; Ullrich et al., 1984). Studies using isolated renal plasma membrane vesicles have demonstrated that the dicarboxylate transport in both brush-border (Kragh-Hansen et al., 1982; Wright, 1985) and basolateral (Wright & Wunz, 1987; Burckhardt, 1984; Lee et al., 1990) membranes is a Na^+-dependent process. The properties of the transport system in the two membranes are similar but the substrate affinity and the transport capacity are different (Kim et al., 1992).

It has been established that a large difference exists in the lipid composition of apical and basolateral membranes originating from the proximal tubule (Hise et al., 1984; Carmel et al., 1985; Molitoris & Simon, 1985). Such a lipid polarity is responsible for the difference between the apical and basolateral membrane fluidity, which can affect transport process in the membrane. Thus, the effect of temperature could differ between the apical and basolateral membrane transport systems.

The present study was carried out to determine 1) whether the effect of temperature is different between the dicarboxylate transport systems of the apical and basolateral membranes, and 2) whether kinetic parameters are altered by temperature. For this study, succinate transport was measured in the brush-bor-

der (BBMV) and basolateral (BLMV) membrane vesicles isolated from rabbit renal cortex over a wide range of temperatures.

MATERIALS AND METHODS

Preparation of plasma membrane vesicles

BLMV and BBMV were simultaneously isolated from New Zealand White rabbits weighing 1.5~2.5 kg of either sex by the Percoll-density gradient centrifugation and Mg^{2+}-precipitation method, as previously described (Kim et al., 1992).

The vesicles were suspended in the vesicle buffer, adjusted to yield a protein concentration of 6 mg/ml and stored at −70℃ until use. Unless otherwise stated, the composition of vesicle buffer was 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Prior to transport studies vesicles were preincubated at 37℃ for 30 min to effectively load with appropriate buffer (Kim et al., 1989).

Transport studies

Uptake of succinate by vesicles was measured by a rapid filtration technique. Briefly, the reaction was initiated by adding membrane vesicles to the incubation medium (a 1 : 10 dilution of membrane vesicle suspension) containing [^{14}C]succinate. The composition of the incubation medium is given in the figure legends. After incubating for 5 sec, 100 μl aliquots were taken and quickly filtered under vacuum through Millpore filters (HAWP, 0.45 μm pore size) which had been soaked overnight in distilled water. The filters were then washed with 5 ml of ice-cold stop solution containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.4) and 0.1 mM $HgCl_2$, and dissolved in 1.0 ml of methoxyethanol. After addition of 10 ml of scintillation cocktail, the amount of radioactivity taken up by vesicles was determined by liquid scintillation spectrometry (Packard Tricarb 300C). Nonspecific binding of substrate to membranes was determined by the same filtration procedure after vesicles were incubated in distilled water containing 0.1% deoxycholate and radioactive substrate.

For temperature studies, the media were equilibrated for 30 min at the appropriate temperature before addition of the membranes. Protein was determined according to Bradford (1976), using γ-globulin as a standard.

Marker enzyme assays

Na^+-K^+-ATPase activity was measured according to Jϕrgensen and Skou (1971). Alkaline phosphatase activity was determined as described by Linhardt and Walter (1963). The enrichment of Na^+-K^+-ATPase, the basolateral membrane marker enzyme, was 18-fold in BLMV and that of alkaline phosphatase, the brush-border membrane marker enzyme, was 14-fold in BBMV.

Calculations and statistical analysis

When requried, the data were analyzed and fitted using the computer program ENZFITTER (Sigma Co, St. Louis, MO, U.S.A.). Student's *t*-test was used in statistical evaluation of the data and p values less than 0.05 were considered significant. All data are expressed as mean ± SE.

Chemicals

[^{14}C]succinate (58.0 mCi/mmol) was purchased from Amersham International (Amersham, UK). Tris and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes) were purchased from Sigma (St. Louis, MO, U.S.A.). Other chemical reagents used were of high purity.

RESULTS

Temperature dependence of succinate uptake

Fig. 1. depicts the temperature dependence of succinate uptake by BBMV and BLMV. When Na^+-dependent succinate uptake was measured in the incubation media with various temperatures between 0° and 40℃, the uptake in-

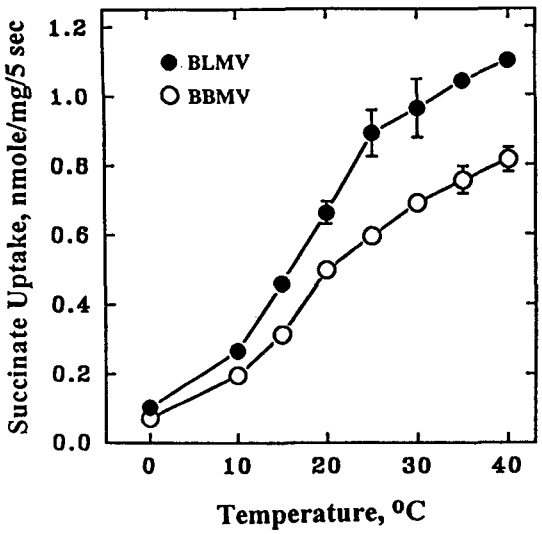

Fig. 1. Temperature dependence of Na^+-dependent succinate uptake in BBMV and BLMV isolated from rabbit kidney. Membrane vesicles were suspended in a buffer containing 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.5), and were incubated for 5 sec in a buffer containing 5 μM [^{14}C]succinate, 100 mM mannitol, 100 mM NaCl and 20 mM Hepes/Tris (pH 7.5). Data are mean± SE of six experiments.

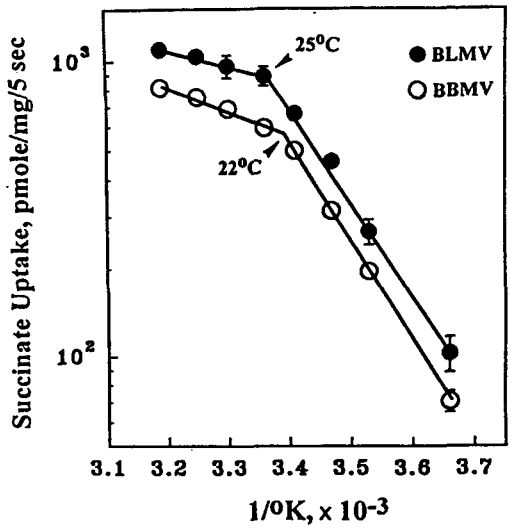

Fig. 2. Arrhenius plots of the data in Fig. 1.

creased with temperature.

In order to evaluate the apparent break point and activation energy, Arrhenius plots were constructed by using the average values of uptake in Fig. 1. The results are illustrated in Fig. 2. For BBMV, a discontinuity occurred in the temperature range betwen 20 and 25℃ and the curve could be fitted into two straight lines, by which a transient tempearture of 22℃ was able to be obtained. For the uptake by BLMV, a similar curvilinear plot was obtained with a break point at 25℃.

The activation energy was estimated using the Arrhenius equation:

$$E = 2.303\ (\log_{10} k_2 - \log_{10} k_1) R / (1/T_1 - 1/T_2)$$

Where k_2 and k_1 are the uptake at the absolute temperature T_2 and T_1, respectively; R is the gas constant, 1.987 cal/degree·mole. When the uptake was plotted as function of the reciprocal of the absolute temperature (Arrhenius plot), the apparent activation energy can be calculated from the slope of the plot. The results of such calculations using Fig. 2 are given in Table 1. The activation energy above the break point temperature was 3.91 and 2.65 kcal/mole for BBMV and BLMV, respectively. The value below the break point was 4~5 times greater than that above the break (17.09 and 14.05 kcal/mole for BBMV and BLMV, respectively).

Kinetic parameters of succinate uptake by BBMV and BLMV

Figs. 3 and 4 show the concentration dependence of succinate uptake at 20 and 35℃ for BBMV and BLMV, respectively. The initial (5 s) rate of succinate uptake was measured as a function of external succinate concentration in the presence of an inwardly directed Na^+ gradient. The uptake increased curvilinearly with the substrate concentration, providing an evidence for saturation. The data were analyzed by using a computerized model of Michaelis-Menten kinetics. The results summarized in Table 2 indi-

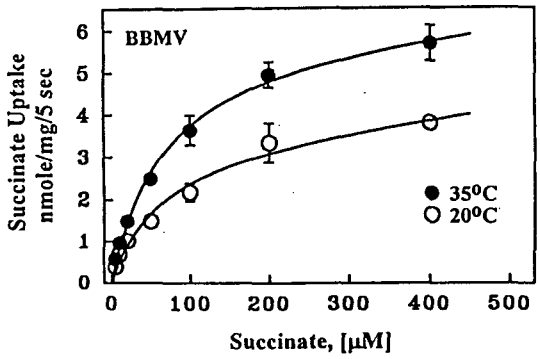

Fig. 3. Effect of temperature on the kinetics of succinate uptake by BBMV. Membrane vesicles were suspended in a buffer containing 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.5), and were incubated for 5 sec in a buffer containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.5) and various concentrations of succinate. Data are mean±SE of six experiments.

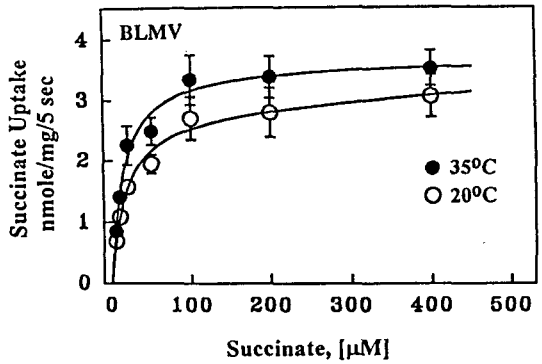

Fig. 4. Effect of temperature on the kinetics of succinate uptake by BLMV. Membrane vesicles were suspended in a buffer containing 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.5), and were incubated for 5 sec in a buffer containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.5) and various concentrations of succinate. Data are mean±SE of six experiments.

Table 1. Temperature dependence of succinate transport systems in BBMV and BLMV

	Break point	Activation energy (kcal/mole)	
		Above break point	Below break point
BBMV	22℃	3.91	17.09
BLMV	25℃	2.65	14.05

Data are mean of six experiments.

Table 2. Effect of temperature on the apparent Km and Vmax of succinate uptake by BBMV and BLMV

	Temperature	Km(μM)	Vmax (nmole/mg protein/5 sec)
BBMV	20℃	62.3±0.032	3.49±0.55
	35℃	65.0±1.47	5.90±0.85*
BLMV	20℃	17.4±3.79	2.86±0.25
	35℃	15.3±3.48	3.63±0.32*

Data are mean±SE of six experiments.
*$p<0.05$ compared to the value at 20℃.

cate that temperature increase from 20 to 35℃ produced a siginficant increase in the Vmax of succinate uptake with no change in Km in both BBMV and BLMV.

DISCUSSION

Epithelial membrane proteins can be classified into two main categories: those sensitive and those not sensitive to the change in membrane physical state. Proteins, such as Na^+-K^+-ATPase of basolateral membrane, and Na^+-dependent D-glucose and phosphate transporters of apical membrane are sensitive to the physical state. By contrast, membrane proteins, such as gamma-glutamyl transpeptidase, leucine aminopeptidase and renal Na^+-alanine cotransport are poorly sensitive to the membrane physical state (Le Grimellec et al., 1992). For membrane proteins sensitive to the physical state of surrounding lipids, the liquid to gel

phase transition is generally associated with large changes in the activation energy evidenced by a "break" in Arrhenius plot. In this study, a clear change in slope in the Arrhenius plot takes place at 22 and 25℃ for BBMV and BLMV, respectively, suggesting that the dicarboxylate transport across the brush border and basolateral membranes is sensitive to a change in the membrane physical state. The transition temperature for BBMV was similar to that for Na^+-Pi cotransport (23℃, Kurnik & Hruska, 1985; De Smedt & Kinne, 1981; Brunette et al., 1984), but was substantially higher than that for Na^+-glucose cotransport (14~19℃, Kinne et al., 1975; Kippen et al., 1979; Kurnik & Hruska, 1985; De Smedt & Kinne, 1981). These results suggest that the lipid environment of the Na^+-dicarboxyate carrier is similar to that of Na^+-phosphate cotransporter but differs from that of the Na^+-glucose cotrans-porter.

In this study, the activation energies below the break point temperature were about 4~5 times higher than those above. In pig renal BBMV, the activation energies are 4.2 and 32.6 kcal/mole for temperature above and below the break point, respectively, for Na^+-glucose cotransport, and the respective value are 8.6 and 24.6 kcal/mole for Na^+-phosphate cotransport (De Smedt & Kinne, 1981). For Na^+-glucose cotransport in rat kidney, the values are 4.2 and 32.6 kcal/mole (Cruz et al., 1983). Thus, the activation energy for Na^+-succinate cotransport in BBMV obtained from this study was lower than that for Na^+-glucose or Na^+-phosphate cotransport.

In this study, a sharp break of the Arrhenius plot at 22 and 25℃ for BBMV and BLMV, respectively, may provide evidence for the existence of a liquid-gel phase transition in both membranes. Experiments using Na^+-K^+-ATPase from rabbit and guinea pig kidneys have demonstrated that the break in the activity of the reconstitued enzyme is associated with a liquid-gel transition of the phospholipid (Kimelberg & Papahadjpoilos, 1974; Palatini et al., 1977). It has also been shown that a break in the Arrhenius plot of Na^+-K^+-ATPase of kidney medulla and cortex corresponds to a change in the membrane physical state (Le Grimellec et al., 1982; Grisham & Barnett, 1973).

In a previous study, we have observed that the succinate transport system of brush border membranes is distinct from that of basolateral membranes in the substrate affinity and transport capacity (Kim et al., 1992). This differernce may be attributed to a difference in lipid composition of the membrane. It has been demonstrated that brush border membranes are much more rigid than basolateral membranes, primarily due to a difference in the lipid composition (Le Grimellec et al., 1982). Experimental data accumulated over the last decade indicate that changes in the lipid composition or physical state affect the transmembrane movement of water and various substances in model and biological membranes (Kimelberg & Papahadjpoilos, 1974). Since temperature induces a change in physical state of membrane, kinetic parametes would be affected by a change in temperature. Brunette et al. (1984) reported that the values of Km and Vmax increase with temperature in phosphate transport by rat renal BBMV. The present study shows that by raising the temperature above the break point the Vmax was increased but Km was not changed, that this trend was similar in both brush-border and basolateral membranes.

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