Membrane Fluidity and Phospholipid Composition in Relation to Sulfur Amino Acid Intake in Brush Border Membranes of Rat Kidney

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ABSTRACT. The transport of ions and solutes across biological membranes may relate to changes in the lipid microenvironment of the membrane which could alter the activity or configuration of transport sites. Changes in the sulfur amino acid content of diets fed to young rats results in an increase in Na⁺-taurine symport in brush border membranes isolated from animals fed a low sulfur amino acid diet and a reduction in symport after a high taurine diet in comparison to uptake by membranes from normally fed animals ("the renal adaptive response"). We explored the possibility that diet-induced changes in brush border membrane symport relate to altered membrane fluidity and phospholipid composition in response to diet. An Arrhenius plot of initial rate (15 s) taurine uptake in breakpoint at 22° C, but no change in relation to dietary alteration. Fluorescence polarization data employing the probe 1,6-diphenyl-1,3,5-hexatriene best fits a two-phase linear model employing a computer model fitting program. Dietary manipulations did not change the breakpoint upper segment slope or lower segment slope after incubating fresh membranes with DPH over the temperature range 4–56° C. No change was evident in membrane phospholipid composition in relation to diet. This study indicates that the changes in initial rate Na⁺-taurine symport in relation to diet are less likely to be due to changes in the configuration of the transporter from an alteration of the lipid microenvironment of the membrane. (*Pediatr Res* 20: 1305–1309, 1986)

Abbreviations

LTD, low sulfur amino acid diet
NTD, normal sulfur amino acid diet
HTD, high taurine diet
DPH, 1,6-diphenyl-1,3,5-hexatriene
tPnA, transpannic acid
TMA-DPH, 1,-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene
BBMV, brush border membrane vesicles
BSA, bovine serum albumin
P, polarization of fluorescence
PC, phosphatidylcholine
SM, sphingomyelin
Lyso PC, lysophosphatidyl-choline
PI, phosphatidylinositol

PS, phosphatidylserine
PE, phosphatidylethanolamine
PG, phosphatidylglycerol
ANOVA, analysis of variance

Renal tubular epithelium is capable of adapting to changes in the dietary intake of various nutrients. This renal adaptive response has been demonstrated for the β -amino acid, taurine, which is conserved following diminished sulfur amino acid intake and hyperexcreted in the urine after dietary taurine excess (1–3). Studies employing isolated luminal brush border membrane vesicles are consistent with these changes in urinary excretion of taurine occurring as the consequence of a membrane-related event (3, 4), since the LTD appears to increase the V_{max} of initial Na⁺-taurine symport and the HTD decrease the V_{max} of uptake into vesicles. We conducted these studies to determine if these diets influence the microviscosity and phospholipid composition of these vesicles.

Changes in the physical status or in the lipid composition of biological membranes can alter the movement of ions and solutes across these membranes (5). The technique of fluorescence polarization has been employed to define the "fluidity" of hepatic, erythrocyte, bacterial cell, gastrointestinal, and renal plasma membranes (6–11). The importance of the lipid microenvironment of biological membranes is also of interest since the activity of membrane-bound enzymes and transport-sites can be influenced by alterations in phospholipid composition (11–13). Lipid-protein interactions may impinge on the activity of proteins located within the membrane (13–16). Hence it is tempting to speculate that the renal adaptive response to alterations in sulfur amino acid intake may be due to changes in membrane fluidity and phospholipid composition in relation to dietary change.

In this study we examine the effect of three diets consisting of varying amounts of sulfur amino acids on membrane fluidity, measured by the use of the fluorescence probe DPH over a wide range of temperatures. In addition the influence of these diets on the phospholipid composition of the apical membrane was assessed. These determinations allow us to indicate if changes in brush border membrane accumulation of taurine are governed by alterations in membrane fluidity or phospholipid compositions.

METHODS

Animals. Male Sprague-Dawley rats (King Animal Labs, Inc., Oregon, WI) aged 58–60 days and weighing 150–180 g were

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employed in all studies. Animals were fasted overnight prior to sacrifice and allowed access to water only.

Diets. The composition of the diet is described in detail elsewhere (3, 4, 17). Briefly each diet (LTD, HTD, or normal taurine diet (NTD)) is isoproteic and contains soy protein which contains limited amounts of methionine and cysteine. The LTD contains 0.2% methionine, the NTD 0.7% methionine, and the HTD 0.7% methionine and 3.0% taurine (w/w). The methionine content for optimal growth is 0.67% of diet as methionine (18). Diets are purchased from ICN, Cleveland, OH. Animals are divided into the three dietary groups and consume the diet for 14 days. Evidence for the adaptive response occurs within 72 h after initiation of diet (17) and is completely established within 6 days. Animals consuming the LTD, which is limiting in methionine and cysteine are $13 \pm 2\%$ smaller at the end of the 14 days (3, 4, 17). No attempts are made to pair-feed these animals since the LTD-fed group actually ingests more food.

Preparation of membranes. BBMVs are prepared according to a modification of the method of Booth and Kenny (19) as completely described previously (4). The final BBMVs fraction was resuspended in 290 mM Mannitol, 10 mM Tris/HEPES (pH 7.4), and either used fresh or frozen at -70°C . Protein was measured according to Lowry *et al.* (20) using BSA as a standard. Determinations were made of membrane γ -glutamyl transferase, 5'-nucleotidase, acid phosphatase (pH 4.8), N-acetyl-B-D-glycosaminidase, succinyl-cytochrome C reductase, malate dehydrogenase, DNA, ouabain-inhibitable $\text{Na}^+ + \text{K}^+$ ATPase (3, 4). Of marker enzymes examined the final membrane preparation contained a 6- to 11-fold increase in specific activity for γ -glutamyl-transpeptidase and 5'-nucleotidase relative to the starting homogenate and a 1.2- to 1.4-fold increase in $\text{Na}^+ + \text{K}^+$ ATPase, a marker of basal-lateral membranes. All other marker enzymes had a lower specific activity indicating excellent enrichment of the brush border fraction.

Uptake studies. The uptake of taurine into brush border membrane vesicles at 23°C was performed and according to our established procedure (3, 4). Uptake is Na^+ and Cl^- -dependent (21) and takes place into an osmotically influenced space, thus accumulation of taurine represents intravesicular uptake (2-4, 21). The internal medium consisted of 278 mM mannitol, 1 mM MgSO_4 , and 10 mM Tris/HEPES (pH 7.4) and the uptake medium (external) consisted of 100 mM NaCl , 1 mM MgSO_4 , 10 mM Tris/HEPES (pH 7.4), and 10 μM taurine with 0.5 μCi of ^3H -taurine. After a given time interval incubated membranes were diluted with a cold (4°C) stop-solution and filtered over a Millipore filter using a cellulose nitrate membrane (HWAP 0.45 μ) after washing three times, membranes were dried overnight, placed in a scintillation vial, submerged in Aquasol, and counted in a β -scintillation counter with background subtraction (3, 4, 21).

Fluorescence polarization. For fluorescence polarization determinations, the lipid soluble fluorescent probe DPH (Aldrich Chemical Co., Inc., Milwaukee, WI) was employed in a 2 mM stock solution in tetrahydrofuran. In a usual experiment, vesicles (2-4 mg protein/ml) in 288 mM mannitol, 10 mM Tris/HEPES pH 7.4, and 1 mM MgSO_4 were incubated with DPH for 15 min at a final concentration of 4 μM . P was measured using an Elscint model MV-1a microviscosimeter (Elscint Inc., Hackensack, NJ). This device displays $P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$ where $I_{\parallel}$ and $I_{\perp}$ represent the fluorescent intensities oriented parallel ($\parallel$) and perpendicular ($\perp$) to the path of polarization of the excitation light (22). Interpretation of the data derived utilizes the Perrin equation, which is written: $r^0/r = 1 + 3\tau/P$, where r^0 is the maximal limiting anisotropy (0.362) for DPH (23), r is the anisotropy, τ is the excited state fluorophore lifetime, and P is the rotational relaxation time. P is usually expressed as the anisotropy parameter $[(r^0/r)-1]^{-1}$, where $r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$. Fluorescence polarization was determined from 4 to 56°C with readings determined every 2°C using freshly prepared BBMVs. The temperature of the vesicle suspension was directly

determined with a digital thermometer (Digitec model 5810) which is accurate to 0.1°C .

There is concern over appropriately drawing the shape of the resulting function when in $[(r^0/r) - 1]^{-1}$ is plotted versus reciprocal absolute temperature, four models were assessed for their ability to fit the data derived. These models were linear, two-phase linear, three-phase linear, and quadratic using linear or quadratic regressions where appropriate. The best two- and three-phase linear models were derived using a computer program based on the theory of Hudson (24) as designed by Gourley *et al.* (25). In order to test the adequacy of each model, residual plots were determined. F tests were performed to indicate the appropriateness of a two-phase compared to a linear model and if a three-phase was superior to a two-phase model. Breakpoints and anisotropy parameters were compared in vesicles prepared from rats on each diet. Mean values were compared between diets using two way analysis of variants.

Phospholipid composition. Extraction and analysis of vesicle phospholipids were accomplished by established methods (26-28). In general, frozen membranes were thawed and a chloroform:methanol (2:1 v/v) extract was prepared and washed free of nonlipid contaminants by the method of Radin (27). Phospholipids were isolated by two-dimensional thin-layer chromatography using an internal standard consisting of ^{14}C -dipalmitoyl PC to measure recoveries for each analysis. Lipid extracts were plated on silica gel H plates; first direction development was performed in a solvent system of chloroform:methanol:water (65:45:4). Plates were then air dried for 10 min, rotated 90° , and developed in tetrahydrofuran:methyl:methanol:water (10:6:4:1). This system allows isolation of PC, SM, Lyso PC, PI, PS, PE, and PG. The method of Bligh and Dyer (26) was used to extract lipids from the gel and phosphorus content was quantitated by the method of Perleman *et al.* (28). The influence of diet on phospholipid composition was compared using Student's t test between groups or ANOVA when indicated.

Supplies. All chemicals used were reagent grade. ^{14}C -dipalmitoyl PC and ^3H -taurine were purchased from New England Nuclear, Boston, MA. DPH was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI.

RESULTS

The uptake of taurine (Na^+ -dependent component) was greater in BBMVs prepared from animals fed the LTD— 34.6 ± 2.6 (SE) pmol/mg protein/15 s—than in NTD vesicles— 18.3 ± 2.1 pmol/mg protein/15 s, $p < 0.001$. Taurine uptake was lower in HTD-fed vesicles at 11.8 ± 1.1 pmol/mg protein/15 s, significantly different from the NTD control, $p < 0.001$ as has been described in our previous studies (3, 4).

When Na^+ -taurine uptake for rats fed the NTD was examined at various temperatures between 4 and 40°C , an Arrhenius plot can be described (Fig. 1). As noted the best computer fit was a two-limb linear model with a break-point at approximately 22° . Break point values after LTD and HTD were 23.1 and 22.8°C , respectively. Thus no alteration in the Arrhenius plot was found despite dietary differences.

P data were assessed by describing Arrhenius-type plots of the log P as a function of reciprocal temperature. Residual plots were determined which allows evaluation of the adequacy of the four models being tested to define the best fit. Using this system, in order for a model to be appropriate, there should be no distinct pattern to the residuals plotted; *i.e.* they should be random. The residual plots for a linear model were parabolic in shape. The two-phase model showed no pattern to its residual plot, whereas a three-phase and quadratic model had marked residual plots. The two-phase linear model also had the lowest residual sum of squares in all 12 data sets. Finally, when F tests were performed to further compare models the two-phase linear model was far superior to the linear or three-phase linear model. A typical plot of log P as a function of reciprocal temperature is shown in

Figure 2. Each figure represents the results of a representative experiment from animals on each of the three diets. The results given in Tables 1 and 2 represent the mean $\pm$ SE of data from all experiments. Thus, the apparent increase in polarization in the LTD animal (Fig. 2A) is not real when all the data available are analyzed.

Table 1 demonstrates transition temperatures or the temperature at which the segmented log P versus reciprocal temperature function abruptly changes slope. The transition temperatures show no significant differences between LTD-, NTD-, or HTD-fed rat brush border membranes (ANOVA). Table 2 shows the values for P and the slopes of the upper and lower segments which represents the apparent energy of activation (10, 11). Again no influence of dietary manipulation on these values was evident.

The phospholipid composition of membranes is displayed in Table 3. The most prevalent phospholipids are SM, PC, PS, and PE regardless of diet. No alteration in the phospholipid profile of the membranes could be associated with the dietary change carried out in this study other than the percentage of glycerol-

PO_4 is slightly but significantly higher in vesicles prepared from rats fed the HTD.

DISCUSSION

As noted in previous studies (2-4) considerable differences in initial rate Na^+ -taurine symport are found in this study in relation to dietary intervention. The LTD results in an almost 2-fold increase in 15-s, taurine uptake by BBMVs and the HTD in a 6% fall in uptake. Because of these substantial differences in Na^+ -taurine symport associated with varying intakes of sulfur amino acids it is tempting to speculate that changes in the lipid microenvironment might underlie these changes.

We first determined the breakpoint of a standard Arrhenius plot and showed that a two phase linear model gave the best fit. The breakpoint determined in membranes from rats fed each of the three diets was similar at approximately 23°C ; thus, a dietary effect is not evident. Changes in physical state of the membrane often correspond to liquid-gel phase transitions in a variety of membranes including the intestine (29, 30). The observation that a clear change in slope in the Arrhenius plot for Na^+ -dependent taurine uptake occurs around 23°C provides evidence that a liquid $\rightarrow$ gel phase transition may be occurring at this temperature. Further, it is similar to the nonlinear Arrhenius plot for alkaline phosphatase in rabbit kidney brush border membranes which occurs at 26°C (10). Whatever are the molecular events responsible for the Na^+ -taurine symport being responsive to changes in the physical state of the membrane lipids, it is clear that the change in the slope of the Arrhenius plot occurs at "room temperature." Hence, it is timely that all experiments were performed within a narrow range around this temperature. Finally, even though this liquid $\rightarrow$ gel transition may occur at a critical temperature for taurine uptake, the dietary manipulations

Fig. 1. The initial rate (15 s) uptake of $10\ \mu\text{M}$ taurine in the presence of an external $100\ \text{mM}$ NaCl gradient in relation to temperature (4 – 56°C). Data are expressed as the reciprocal of the absolute temperature, an Arrhenius plot. Each point represents the mean of five separate experiments determined in triplicate. The plot was fit to a 2-phase linear model using the program established by Gourley *et al.* (25).

Table 1. P data as function of reciprocal temperature (K°) in brush border membranes from rats in each diet

Diet	K° *	Statistics	
LTD	25.3 ± 1.1	LTD vs NTD	NS
NTD	23.5 ± 0.6	NTD vs HTD	NS
HTD	23.6 ± 0.1	LTD vs HTD	NS

* Temperature $\pm$ SE of five separate experiments expressed as $^\circ\text{C}$. Statistics by ANOVA.

Fig. 2. Computer derived plots of membrane polarization data derived in relation to inverse absolute temperature. A, low taurine diet; B, normal diet; C, HTD. Each point is a representative graph of one of four determinations in membranes prepared from animals on each diet.

Table 2. Values for *P* and slopes of upper and lower segments

	LTD	NTD	HTD	LTD vs NTD	NTD vs HTD	LTD vs HTD
P	0.3366* ±0.0106	0.3341 ±0.0080	0.3342 ±0.0060	NS	NS	NS
Upper segment	353.0 ±9.1	320.33 ±23.13	341.17 ±2.85	NS	NS	NS
Lower segment	204.88 ±32.10	170.88 ±16.51	196.84 ±12.49	NS	NS	NS

* Mean ± SE of five determinations at each point. Statistics by ANOVA.

Table 3. Phospholipid composition of brush border membranes*

	LTD	NTD	HTD	LTD vs NTD	NTD vs HTD	HTD vs LTD
Lyso PC	6.86 ±2.18	6.78 ±2.18	8.20 ±1.88	NS	NS	NS
SM	82.60 ±4.30	82.27 ±2.30	88.23 ±5.78	NS	NS	NS
PC	71.97 ±8.46	62.48 ±12.05	78.84 ±2.47	NS	NS	NS
PS	113.41 ±6.70	133.49 ±11.01	113.67 ±7.18	NS	NS	NS
PI	3.65 ±0.47	2.50 ±1.01	2.01 ±0.55	NS	NS	NS
PE	63.46 ±5.72	60.39 ±0.78	67.79 ±6.12	NS	NS	NS
Glycerol phosphate	5.12 ±0.55	1.27 ±0.59	1.55 ±0.65	<0.05	NS	<0.05

* $\mu\text{mol Pi/mg}$; mean ± SE of four determinations. Statistics by ANOVA.

that we have employed do not alter this change occurring at "room temperature."

In terms of DPH fluorescence with regard to temperature, the two-phase linear model gave the most appropriate fit. Again regardless of diet, the transition temperature at which the segmented log *P* in relation to reciprocal temperature abruptly changed slope as similar. Since the values for *P* and the slopes of the upper and lower segment were also uninfluenced by diet, changes in the physical composition, and membrane fluidity (11–15), at least as measured by the fluorescent probe DPH, do not appear to play a role in the renal adaptive response expressed at the apical membrane of the proximal tubule.

Nonuniform distribution of membrane components raises some degree of uncertainty regarding the lipid microenvironment "viewed" by only one fluorescent probe. Investigators have employed in addition to DPH, such probes as tPnA (10) or TMA-DPH (31). Herein we measured fluidity with only a single probe, DPH, since several of these other probes did not appear to provide a great deal more information. The fluorescence anisotropy and transition temperatures found in rabbit renal brush border membranes were almost identical when DPH and tPnA were used in parallel (10). The probe TMA-DPH may be less sensitive in rat renal brush border membranes (31) and, in general, is a less sensitive probe (32). Furthermore, the probe DPH is less influenced by changes in ionic composition (23). Finally since we found no change in transition temperature, *P*, and in apparent energy of activation in relation to dietary manipulation using DPH as a probe, we purposely chose not to examine other probes. Nonetheless, since we have employed only one probe in *P* experiments, we cannot be certain that the changes in diet might not cause a change in the lipid environment of the brush border membrane probed by other compounds.

As a further measure of the lipid microenvironment, we measured the phospholipid composition of the membranes. As indicated in Table 3, the only change noted in relation to diet was a higher glycerol phosphate value in membranes from LTD-fed

animals. Nonetheless this difference was relatively minor in relationship to other phospholipids which were far more prevalent in the membrane such as PC, PS, and PE. Other groups (10, 11) have also reported high levels of these phospholipids in dog and rat BBMVs although minor differences in the relative concentrations of each phospholipid species were found between the observations herein and those reported by Molitoris and Simon (11).

It may not be readily apparent why manipulation of amino acids in the diet could, at least theoretically, result in a change in the phospholipid composition of the membrane. One of the main factors that can affect the status of lipids within the plasma membranes is the potential for oxidant stresses within the environment. An important protective system which prevents peroxidation of membrane-bound lipids is reduced glutathione (39). A reduction in the extent of unsaturation of the acyl chains of phospholipids will increase membrane fluidity, lead to phase separations in the lipid domain, and enhance enzyme activity (10, 39). The LTD contains limiting quantities of methionine and cysteine which could potentially limit the availability of cysteine for glutathione synthesis and high levels of taurine are known to protect against oxidant stresses (34). Even though we have demonstrated that the glutathione-oxidizing compound, diamide, has no influence on taurine uptake by BBMVs (35), the potential for alterations in the diet with reference to sulfur amino acids to alter membrane phospholipid composition by the mechanism of reduced protection against oxidant stress is worthy of investigation. After examining the influence of the three diets on phospholipid composition and on membrane fluidity, this mechanism does not seem likely.

In summary, this study fails to show any change in Arrhenius plot, membrane fluidity, or phospholipid composition as underlying the change in Na^+ -taurine symport brought about by alterations in the sulfur amino acid composition of the diet. Since the change in symport is expressed as a change in V_{max} of initial rate uptake (1–4), an alteration in the amount of Na^+ -taurine

transporter is more likely to be the mechanism of the adaptive response rather than a change in the configuration of the transporter. Nonetheless, the present study is limited in that it examines only a single probe of membrane fluidity, and has not examined fatty acid composition of these phospholipids. Accordingly, it is difficult to make a definitive statement regarding the lipid environment around the Na⁺-taurine transporter.

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