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Static and Dynamic Components of Renal Cortical Brush Border and Basolateral Membrane Fluidity: Role of Cholesterol

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Summary. Static polarization and differential polarized phase fluorimetry studies on rat renal cortical brush border (BBM) and basolateral membranes (BLM) were undertaken to determine the membrane components responsible for differences in BBM and BLM fluidity, whether these differences were due to the order or dynamic components of membrane fluidity and if a fluidity gradient existed within the bilayer. Surface membrane proteins rigidified both BBM and BLM fluidity. Neutral lipid extraction, on the other hand, caused a larger decrease in BBM than BLM fluorescence polarization (0.104 *vs.* 0.60, $P < 0.01$) using diphenyl hexatriene (DPH). Cholesterol addition to phospholipid fractions restored membrane fluidity to total lipid values in both BBM and BLM phospholipids. The response to cholesterol in the BBM was biphasic, while the BLM response was linear. Lateral mobility, quantitated using dipyrenylpropane, was similar in both BBM and BLM fractions at 35°C. BBM and BLM differed primarily in the order component of membrane fluidity as DPH-limiting anisotropy (r_∞) (0.212 *vs.* 0.154, $P < 0.01$) differed markedly between the two membrane fractions. The two membrane components also differed with respect to 2 and 12-anthroyloxy stearate (2-AS, 12-AS) probes, indicating a difference in the dynamic component of membrane fluidity may also be present. DPH and 12-AS probes were also used to quantitate inner core membrane fluidity and showed the BBM was less fluid than the BLM for intact membranes, total lipid extracts and phospholipids. Results obtained using the surface membrane probes trimethylammonium-DPH (TMA-DPH) and 2-AS suggested a fluidity gradient existed in both BBM and BLM bilayers with the inner core being more fluid in both membranes. These data indicate cholesterol is in large part responsible for fluidity differences between BBM and BLM and that these membranes, while clearly differing in the order component of membrane fluidity, may also differ in the dynamic component as well.

Key Words renal · brush border membranes · basolateral membranes · fluorescence polarization · cholesterol

Introduction

With increasing frequency it is being recognized that the physical state of the membrane has major biological importance. The physical state of membrane lipids has been shown to influence such membrane enzymes as NaK-ATPase [7], hormone-responsive adenylate cyclase [8], and membrane transport processes such as glucose and amino acid uptake [1, 6]. Membrane lipids also play an important role in membrane permeability to sodium, calcium, and potassium [9].

Numerous techniques have been developed to quantitate the physical state of the membrane. The term "membrane fluidity" has evolved to express this physical state, and a useful functional definition of membrane fluidity is the selective motional freedom of lipid molecules within the bilayer [22]. Membrane fluidity is determined by several factors including the fatty acyl chain itself, phospholipids, cholesterol, proteins, temperature, and pressure [22–25, 28, 29]. A potential problem in using the term "membrane fluidity" is that it actually consists of two major components. These components may change independently and to date there is no reliable means of calculating one from the other. The first component is the order parameter (S), which is a measure of the angular range of rotational motion. This parameter is also known as the static, structural, or range component of membrane fluidity and relates to the packing of individual fatty acyl chains within the bilayer. The second major component is referred to as either the rate, microviscosity or dynamic component of membrane fluidity and measures the rate of rotational motion, which is a reflection of membrane microviscosity. This component of membrane fluidity may be more important in determining lateral mobility and intramembranous protein coupling interactions [13].

Renal cortical brush border (BBM) and basolateral membranes (BLM) provide an excellent model to study the effect of membrane components on the physical state of the membrane as there are large differences in the lipid composition of BBM and BLM [4, 11, 19]. This is especially true with respect to sphingomyelin (34.5 *vs.* 13.4%), phos-

phatidylcholine (16.2 *vs.* 38.4%) and the cholesterol to phospholipid ratio (0.9 *vs.* 0.5), all of which are major factors influencing membrane fluidity. Differences in renal cortical brush border and basolateral membrane fluidity were first described by Le Gerimellac et al. [15]. Using steady-state fluorescence polarization and electron-spin resonance, they demonstrated in dog kidney membranes that the BLM was much more fluid than the BBM. Brush border and basolateral membranes isolated from human renal cortical segments [14] gave similar results. Hise et al. [10], again using steady-state fluorescence polarization and electron-spin resonance, showed similar findings in rat renal cortical brush border membranes. These authors observed a larger quantity of saturated fatty acids in the brush border membrane. In a subsequent study [11] this was shown to be due to the high content of sphingomyelin in brush border membranes; fatty acyl chain saturation, but not chain length was shown to influence renal cortical brush border membrane fluidity. The authors concluded that the sphingomyelin-to-phosphatidylcholine ratio was an important determinant of renal membrane fluidity by virtue of the high content of saturated fatty acids present in sphingomyelin. Cholesterol content, however, has also been proposed as the major determinant of differences between BBM and BLM fluidity [5]. The present studies, therefore, were undertaken to determine the role of cholesterol in determining the differences between renal cortical BBM and BLM fluidity. We also factored BBM and BLM fluidity into static and dynamic components and investigated whether a fluidity gradient exists within BBM and BLM bilayers.

Materials and Methods

Preparation and Characterization of Membranes

Male Sprague-Dawley rats (200–280 g) maintained on standard chow were used in all experiments. Renal cortical BBM and BLM were isolated simultaneously from the same cortical homogenate and characterized enzymatically as described in detail elsewhere [18, 19]. Briefly, the procedure entailed rapid decapsulization and removal of thin cortical slices in chilled buffer [300 mM mannitol, 5 mM ethyleneglycol-bis (*b*-aminoethylether)-N, N′-tetraacetic acid, 18 mmol Tris (hydroxymethyl) aminomethane hydrochloride, 0.1 mM phenylmethylsulfonyl fluoride at pH 7.4]. The slices from two kidneys were homogenized using a Polytron PT 200D (Brinkman) in 15 ml of buffer, and Mg^{2+} precipitation (15 mmol/liter) was carried out for 20 min. The resulting solution was centrifuged for 15 min at 2,445 × *g*, the pellet (P_2) was saved for BLM isolation, while the supernatant was centrifuged at 48,000 × *g* for 30 min to obtain crude apical membranes. This pellet was resuspended using a Potter-Elvehjem homogenizer in 30 ml of diluted buffer (1 : 1 with deionized water) and taken through the Mg^{2+} precipitation process again.

To isolate the BLM, fraction P_2 was resuspended using a loose Dounce glass homogenizer followed by repeat addition of Mg^{2+} (15 mM) and then centrifuged at 2,445 × *g* for 15 min. The pellet (P_3) was resuspended in standard buffer, diluted with water (1 : 1), and centrifuged for 15 min at 775 × *g*. The supernatant was centrifuged for 30 min at 48,000 × *g*, the resulting pellet (P_5) was resuspended in 19 ml of 50% sucrose overlaid with a discontinuous sucrose gradient using 41% (5 ml) and 38% (12 ml) in cellulose-acetate tubes centrifuged at 88,000 × *g* at 4°C in a Beckman Model L8-70 Ultracentrifuge for 3 hr, and the top layer (38%) was harvested. In BBM fractions alkaline phosphatase enrichment was 12.2 ± 3.4 while NaK-ATPase was enriched 1.5 ± 0.7-fold. In BLM fractions NaK-ATPase enrichment was 9.1 ± 3.3 while alkaline phosphatase was only enriched 1.5 ± 0.6-fold over homogenate values. These BBM and BLM marker enzyme enrichments are consistent with previous studies [18–20] from this laboratory.

Enzyme and Protein Measurements

Protein was measured according to Lowry et al. [24] using bovine serum albumin as a standard. Enzyme determinations were carried out using standard kinetic assays as previously reported from our laboratory [18, 19].

Fluorescence Polarization Measurements

Steady-state fluorescence polarization studies were conducted as we have previously described [18]. Briefly, a HH-I T-format polarization spectrofluorimeter (BHL Associates, Burlingame, CA) with fixed emission and excitation polarization filters was used to quantitate fluorescence intensity perpendicular ($I_\perp$) and parallel ($I_\parallel$) to the polarization phase of the exciting light [23]. Polarization of fluorescence $[(I_\parallel - I_\perp)/(I_\parallel + I_\perp)]$ was calculated by an online microprocessor. The excitation wavelength was 362 nm, a 03FCG001 filter (Melles Griot, Irvine, CA) was used for the excitation beam, and KV389 filters (Schott Optical Glass, Duryea, PA) were used for the emitted light. BBM and BLM samples (72 μg protein) were brought to total volume of 1.2 ml with phosphate-N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid-buffered saline (PBS) [18], and fluorescent probes were incorporated at 35°C for 10–20 min under argon with frequent vortexing. Values for polarization were recorded at 35°C. Probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), were dissolved in tetrahydrofuran (THF) and THF-water (1 : 1), respectively, to give a final concentration of 0.01 μg/ml. Anthroyloxy stearate probes, 2-(9-anthroyloxy) stearic acid (2-AS) and 12-(9-anthroyloxy) stearic acid (12-AS) were also dissolved in THF to give final concentration of 0.05–0.2 μg/ml. The excitation wavelength was 363 nm. Total fluorescence for the AS probes averaged 8.6 ± 2.6 and 9.8 ± 4.5 for 2-AS and 12-AS, respectively, and values for BBM and BLM fractions were not different. The conjunguated pyrene probe 1,3-di(1-pyrenyl) propane was dissolved in THF, and 0.15 nmol of probe was added to each sample. The excitation wavelength was 345 nm, ad emission was measured at 395 and 490 nm for the monomer and

excimer, respectively. The 395/490 ratio was used as a measure of monomer-to-excimer concentrations. Probes were purchased from Molecular Probes (Junction City, OR). Assuming 100% incorporation of probes into the lipid phase, the approximate lipid/probe ratios were 1000:1, 2000:1 and 500:1 for DPH, TMA DPH and the AS probes, respectively.

Differential polarized phase fluorimetry studies were conducted on an SLM 4800 spectrofluorimeter. Fluorescent polarization (P), lifetimes (τ) and differential tangents ($\tan\Delta$) were measured by phase and modulation techniques at modulation frequencies of 6 and 30 MHz [13]. Fluorescence lifetimes were measured with the excitation polarizer set at zero and the emission polarizer set at 55°. Lifetimes were quantitated relative to a reference solution of DPH in hexadecane which has a known lifetime of 9.6 nsec [3]. Heterogeneity analysis was performed using software supplied by SLM and revealed a best fit for a single exponential decay curve in all cases. From these determinations the rotational correlation time (R) and limiting fluorescence anisotropy (r_∞) were calculated according to Lakowicz [13]. The order parameter (S) was calculated where $S = (r_\infty/r_o)^{\frac{1}{2}}$ with r_o taken as 0.365 for DPH [28, 29].

Separation of Membrane Components

To obtain total extracted lipids, the procedure of Molitoris et al. [18] was used. An aliquot of BBM sample containing 500–600 μg of protein was pelleted, and the supernatant was removed as completely as possible. The pellet was resuspended in 0.5 ml of methanol and transferred to a Teflon-capped tube. The microfuge tube was washed with an additional 0.5 ml of methanol, and the two solutions were combined. After addition of an equal volume of chloroform (1 ml), the tube was vortexed and stored under nitrogen at −20°C for 1–2 hr. Membrane protein and particulate matter were separated by using low suction through a Whatman GF/C filter, and the filter and particulate material were then washed with 1 ml of a 2:1 methanol-to-chloroform solution to insure complete extraction. The extracted lipid solution was taken to dryness using nitrogen and resuspended in a known volume of chloroform. An aliquot was saved for fluorescence polarization, and the remainder was used for separation into neutral lipid and polar lipid fractions.

A 1–1.5 g heat-activated (120°C for 2 hr) silicic acid column was used to fractionate the total lipid extract into neutral lipids and polar lipids. The sample suspended in chloroform was added to the column, and the column was washed with 20 ml of chloroform to remove the neutral lipids. Methanol (20 ml) was then used to remove the polar lipids from the column. No cholesterol could be detected by our gas liquid chromatography method in the polar lipid fraction. In addition, the recovery of phospholipids from the column was >95%. The neutral and polar lipid fractions were taken to dryness using nitrogen and resuspended in known volumes of chloroform.

Steady-state fluorescence polarization measurements were carried out on multilamellar vesicles prepared from total lipid fractions. The fluorescent probes were added to the lipid extracts, the resulting solution was dried under a stream of nitrogen, and vesicles were formed in PBS by vortexing and sonication, as described previously [18]. The amount of total lipid extract of polar lipid fraction used was calculated to be equivalent to the amount contained in 72 μg of initial membrane protein. Cholesterol addition to phospholipid fractions was made just prior to nitrogen evaporation using a concentrated chloroform solution.

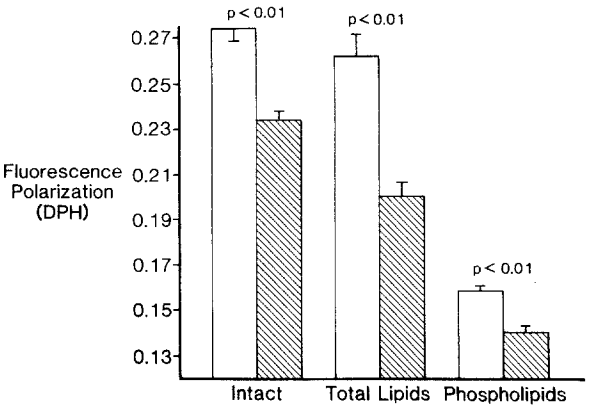

Fig. 1. Differences in DPH fluorescence polarization for intact membranes, total lipid fractions and phospholipid fractions from BBM (open bars) and BLM (hatched bars). Differences between intact and total lipid fractions are due to selective protein removal. The decrease in polarization between total lipids and phospholipid fractions is due to selective neutral lipid extraction. Means ± SD, n = 5 or 6

Statistical Analysis

A paired 2-tailed Student's t test was used to determine statistical significance unless otherwise indicated. Values were considered significantly different if the P value was ≤0.05 and are reported as NS, < 0.05 and 0.01. All values are reported as the mean ± SD unless otherwise noted, and n refers to the number of individual preparations.

Results

To quantitate the role various membrane constituents play in determining the differences in BBM and BLM membrane fluidity, selective extraction procedures were carried out. In Fig. 1, the effect of proteins, neutral lipids (primarily cholesterol) and phospholipids on DPH fluorescence polarization are shown. For BBM, removal of the membrane proteins had a small but statistically significant effect (0.277 ± 0.006 *vs.* 0.262 ± 0.009, $P < 0.05$) on DPH polarization values. Removal of BBM neutral lipids, however, caused a marked reduction in BBM fluorescence polarization values (0.262 ± 0.009 *vs.* 0.158 ± 0.002, $P < 0.01$). For BLM removal of the protein fraction produced a large (0.234 ± 0.004 *vs.* 0.199 ± 0.006, $P < 0.01$) reduction in fluorescence polarization values. These data are in agreement with previous studies, indicating the protein component of the BLM has a larger influence on membrane fluidity [10, 15]. Removal of BLM neutral lipid fraction produced a significant reduction in fluorescence polarization (0.199 ± 0.006 *vs.* 0.139 ± 0.004, $P < 0.01$), although the magnitude of the reduction was significantly less than that seen for

BBM (0.104 *vs.* 0.060, $P < 0.01$). On a relative basis the reduction in BBM and BLM DPH polarization upon neutral lipid extraction was 40 and 30%, respectively. Finally, it should be noted that native BBM phospholipids had a higher fluorescence polarization than BLM phospholipids (0.158 ± 0.002 *vs.* 0.139 ± 0.004, $P < 0.01$).

The 12-AS probe (Table 1) gave similar results. Removal of both protein and neutral lipid fractions resulted in significant reductions in both BBM and BLM fluorescence polarization. Again, removal of the neutral lipid fraction resulted in a larger decrease in fluorescence polarization (0.027 *vs.* 0.012, $P < 0.01$) in the BBM fraction. The results with the superficial probe 2-AS are also shown in Table 1. In counter distinction to the inner core probes, BLM superficial fluorescence polarization was unaffected by removal of the protein and neutral lipid fractions ($P > 0.05$). Again, however, there was a difference between BBM and BLM fluorescence polarization ($P < 0.01$) for intact membranes, total lipids and phospholipid fractions.

Table 1. Fluorescence polarization of renal cortical brush border and basolateral membranes using anthroyloxy stearate probes

	2-AS		
	Intact	Total lipids	Phospholipids
BBM	0.173 ± 0.004	0.159 ± 0.006	0.136 ± 0.002
BLM	0.133 ± 0.007	0.134 ± 0.004	0.127 ± 0.001
P Value	<0.01	<0.01	<0.01
	12AS		
BBM	0.139 ± 0.006	0.104 ± 0.002	0.077 ± 0.003
BLM	0.115 ± 0.002	0.081 ± 0.004	0.069 ± 0.004
P Value	<0.01	<0.01	<0.01

Values represent the mean ± 1 SD, $n = 4 - 6$. 2-AS, 2-(9-Anthroyloxy) stearic acid; 12-AS, 12-(9-anthroyloxy) stearic acid.

To determine if the large reductions in fluorescence polarization following neutral lipid extraction were due primarily to removal of membrane cholesterol, studies adding cholesterol to BBM and BLM phospholipid fractions were conducted. The results of these studies are shown in Fig. 2. Clearly, adding cholesterol to both BBM and BLM phospholipids increased their respective fluorescence polarization. For the BLM a linear effect was seen throughout the range of cholesterol addition. When cholesterol was added to a cholesterol/phospholipid ratio of 0.5, a ratio similar to intact BLM [18, 19], fluorescence polarization returned to values similar to those found for total extracted lipids (0.185 ± 0.008 *vs.* 0.199 ± 0.006).

For the BBM, cholesterol addition resulted in a statistically biphasic response ($P < 0.01$) as determined using breakpoint analysis [12]. Initially, there was a rapid increase in fluorescence polarization with a slope of 0.159. This was followed by a much slower rate of rise (0.049) that was not different from the BLM slope. Again, addition of cholesterol to a cholesterol/phospholipid level normally found in the BBM (0.9) restored fluorescence polarization to values found in total extracted lipid fractions (0.252 ± 0.010 *vs.* 0.262 ± 0.009). These data indicate cholesterol has a major influence on inner core fluidity and is responsible for the large decreases in fluorescence polarization when neutral lipids are removed.

Studies were then undertaken to evaluate the lateral mobility or diffusibility in BBM and BLM. To accomplish this the pyrene probe dipyrenylpropane was used [22]. The results of these studies are shown in Fig. 3. At 27 and 31°C a significant difference ($P < 0.05$) existed with BBM having less lateral movement. As temperature continued to be increased, however, differences between the two membranes became smaller and at 35°C there was no longer a statistically significant difference between the two membranes.

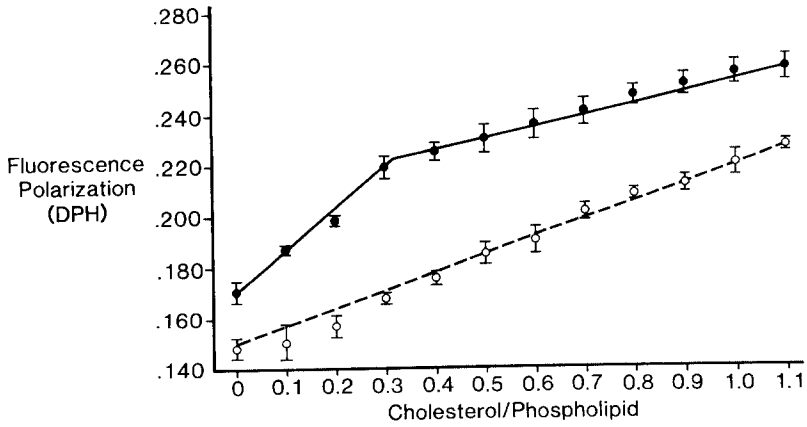

Fig. 2. Response in BBM (—●—) and BLM (--○--) phospholipid polarization (DPH) to addition of cholesterol. Linear regression and break-point analysis was carried out according to Jones and Molitoris [12] and a statistically significant ($P < 0.01$) break point was found for BBM phospholipids. Mean ± SE, $n = 4$

Differential polarized phase fluorimetry studies were then used to factor membrane fluidity into its two components. The data in Table 2 show that BBM and BLM differ in respect to fluorescent lifetimes, total and limiting anisotropy but not rotational correlation times. However, the high variability in rotational correlation times may have masked a difference between the two membranes. The calculated order parameters (S) for the BBM and BLM were 0.762 and 0.650, respectively. Taken together these later two studies indicate that at near physiological temperatures (35°C), the differences in BBM and BLM steady-state fluorescence polarization are due primarily to differences in the order component of membrane fluidity, although a difference in the dynamic component may also be present.

Differences in BBM and BLM inner core and surface membrane fluorescence polarization, as measured by DPH and TMA-DPH, respectively, were then determined and are shown in Fig. 4. Brush border membranes had a higher fluorescence polarization (less fluid) with the inner core probe DPH (0.277 ± 0.006 *vs.* 0.234 ± 0.004, $P < 0.01$). Surface membrane fluidity, measured with the superficial bilayer probe TMA-DPH, was also less fluid in BBM fractions (0.335 ± 0.004 *vs.* 0.324 ± 0.005, $P < 0.01$). In addition, the lower fluorescence polarization value for DPH, as compared to TMA-DPH, in both BBM and BLM fractions is consistent with the inner core portions of both membranes being more fluid.

To verify these findings a second group of probes was used. Anthroyloxy stearate probes, with the emitting molecule on the 2 and 12 carbon positions, were also used to quantitate the fluidity gradient at different levels within the bilayer. Data from 2-AS and 12-AS in intact BBM and BLM are shown in Table 1. Again, intact BBM, relative to BLM, were less fluid for both the inner core probe 12-AS (0.139 ± 0.006 *vs.* 0.115 ± 0.002, $P < 0.01$) and the more superficial probe 2-AS (0.173 ± 0.004 *vs.* 0.133 ± 0.007, $P < 0.01$). Using this family of probes the inner core portion of the bilayer again appeared more fluid for both BBM and BLM fractions ($P < 0.01$).

Discussion

Recently several different laboratories have shown that renal cortical brush border and basolateral membranes differ markedly with respect to steady-state fluorescence polarization. Investigation into the reasons for these differences, however, has led to different conclusions. Le Gerimellac et al. [15] showed that the differences between the two membranes were not due to their protein composition and postulated cholesterol was the primary determinant [5]. Hise et al. [11] suggested that saturated

Table 2. Differential polarized phase fluorimetry of DPH in renal cortical brush border and basolateral membranes

		Anisotropy		
	T (nsec)	Total	r_∞	R
BBM	10.8 ± 0.2	0.226 ± 0.002	0.212 ± 0.003	0.190 ± 0.017
BLM	9.3 ± 0.1	0.177 ± 0.002	0.154 ± 0.001	0.174 ± 0.009
P Value	<0.01	<0.01	<0.01	NS

Values represent the mean ± SD, $n = 6$. T, fluorescence lifetime; r_∞, limiting fluorescence anisotropy; R, rotational correlation time. Fluorescence lifetimes were measured at 30 MHz.

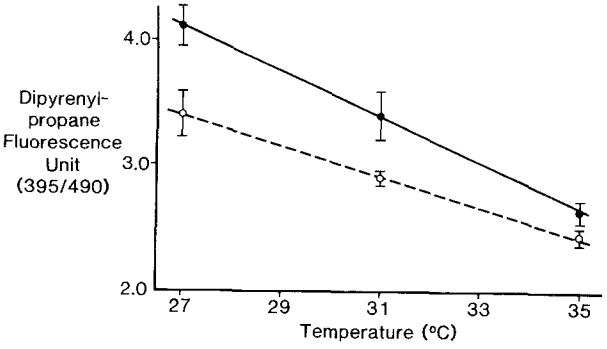

Fig. 3. Ratio of monomer to excimer dipyrenylpropane emission. Fluorescence for BBM (open symbols, $n = 5$) and BLM (filled symbols, $n = 6$) total extracted lipids at varying temperatures. Emission at 395 and 490 was used to quantitate monomer and excimer emissions, respectively. Means ± SE

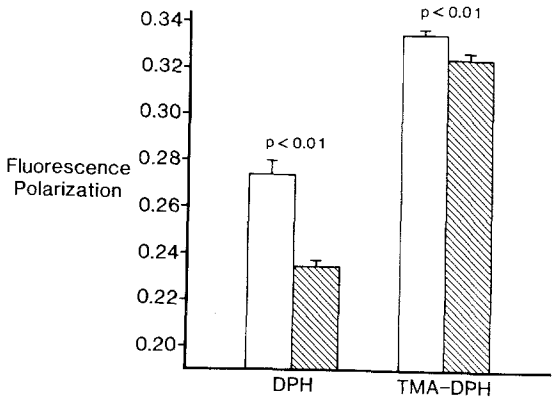

Fig. 4. Differences between BBM (open bars) and BLM (hatched bars) with respect to DPH and TMA-DPH fluorescence polarization. Means ± SD, $n = 6$

fatty acyl chains play an important role in making the brush border membrane more rigid.

Our primary goal, therefore, was to determine the importance of different membrane constituents in determining the large differences in brush border and basolateral membrane fluidity. To do this, selective extraction procedures were used. The change in fluorescence polarization following a membrane components removal was then determined and served as a measure of the component's influence. Protein removal from both brush border and basolateral membranes significantly affected inner core membrane fluidity, as determined by DPH and 12-AS. This was also true for the superficial probe 2-AS for brush border membranes but not basolateral membranes. These data indicate that proteins play a significant role in determining renal cortical brush border and basolateral membrane fluidity, but changes were similar in both membrane fractions with the exception of the basolateral membrane and the superficial probe 2-AS. Therefore, proteins do not account for the large differences in fluorescence polarization between the two membrane fractions, which is in agreement with earlier work [10, 15].

To evaluate the role of cholesterol, we undertook a selective extraction of neutral lipids. This procedure quantitatively removed all of the neutral lipids, which primarily consisted of cholesterol, leaving only phospholipids. We then used the same probes to again measure fluorescence polarization in the remaining phospholipid fraction. The results shown in Fig. 1 and Table 1 indicate that removal of neutral lipids reduced fluorescent polarization in brush border membranes to a greater extent than in basolateral membrane regardless of whether an inner core or superficial fluorescent probe was used to monitor membrane fluidity. This is consistent with other data showing that the brush border membrane contains a much larger quantity of cholesterol and a much higher cholesterol-to-phospholipid ratio [5, 18, 19]. Further evidence, implicating cholesterol was primarily responsible for these dramatic changes, came from cholesterol-reconstitution experiments. In these studies known quantities of cholesterol were added to native BBM and BLM phospholipids. Cholesterol readdition to the phospholipid fraction restored membrane fluidity to the state observed prior to neutral lipid fraction extraction. The reason for the biphasic response to cholesterol readdition in BBM phospholipid polarization was not determined in these studies. However, this could have been due to the strong interactions between sphingomyelin and cholesterol [2] as the BBM contains large amounts of sphingomyelin [18–20]. Cholesterol, therefore, seems to be primarily responsible for the large reduction in fluorescence polarization when neutral lipids are extracted. The data also suggest that the difference between brush border and basolateral membrane fluorescent polarization is due in large part to the differences in cholesterol content between the two membranes. Differences in BBM and BLM cholesterol content have been found by some investigators [5, 19] but not others [4, 10]. The reason for this discrepancy is unknown.

Finally, in all cases brush border membrane phospholipids had a higher fluorescence polarization, using any of the probes, than basolateral membrane phospholipids. This is in agreement with the work previously reported by Hise et al. [11], that native brush border membrane phospholipids are less fluid than basolateral membrane phospholipids. That a difference between the two phospholipid membrane fractions remains significant indicates factors other than cholesterol are also important in determining differences between BBM and BLM fluidity. In our membrane lipid separation scheme glycolipids would also be present in the methanol eluded or phospholipid fraction. The role that differences in BBM and BLM glycolipids play in determining membrane fluidity was, however, not investigated.

Three different approaches were used to determine whether the marked differences in renal cortical brush border and basolateral membranes fluorescence polarization were due to the order or dynamic components of membrane fluidity. This may have physiologic importance as the order component relates to molecular packing and the dynamic or microviscosity component relates more to processes involved with lateral diffusion or intramembranous coupling. First, a pyrene derivative, which has successfully been used to quantitate lateral mobility within the membrane [22, 32, 33], was used to determine the extent of lateral mobility in renal cortical brush border and basolateral membranes. Using this probe one monitors emission of both the monomer (M) and excimer (E) compounds in such a way that a ratio can be derived and used as a measure of lateral mobility [22, 32, 33]. As probe mobility within a membrane increases emission from the excimer predominates since formation of the intramolecular excimer is dependent upon lateral movement of its two components. Therefore, a reduction in the M/E ratio is an indication of increased lateral mobility of the probe within the membrane. For both membranes as temperature increased from 27 to 35°C the ratio decreased, indicating more lateral mobility of the probe within both membranes. At low temperatures (27 and 31°C) there was a statistically significant difference between the two membranes with the basolateral membrane showing more lateral mobility. As physi-

ologic temperatures were approached, however, the probe behaved similarly in both membranes, indicating that at physiologic temperatures brush border and basolateral membrane have similar lateral mobility characteristics.

Secondly, differential polarized phase fluorimetry was used as it allows direct measurements of lifetimes, anisotropy and differential tangents. From these determinations the rotational correlation time and limiting fluorescence anisotropy can be calculated [13]. Brush border and basolateral membranes, again, differed in total anisotropy values. The basolateral membrane also had a statistically significant shorter half-life. There was also a significant difference in the limiting anisotropy between the brush border and basolateral membrane with the brush border being less fluid. Limiting anisotropy represents the structural or order component of membrane fluidity and is in agreement with the differences in calculated order parameter (S) for BBM and BLM. The rotational correlation time (R) represents that component of membrane fluidity due to the dynamic or rate component and there was no statistically significant difference between the two membranes. Rotational correlational time quantitation, however, was more variable and a trend did exist, suggesting a difference may be present between the two membranes.

Our third approach involved use of the AS probes. These probes have been suggested to measure primarily the dynamic component of membrane fluidity [22, 31]. Differences of opinion, however, exist on this issue [26, 27], and these differences may relate to membrane cholesterol content [26, 27] and the excitation wavelength used [31]. Using both the 2 and 12-AS probes, we found differences between BBM and BLM for intact membranes, total lipid and phospholipid fractions, suggesting BBM and BLM may also differ in the dynamic component of membrane fluidity. Taken together, these data indicate that the large differences between renal cortical brush border and basolateral membranes, consistently observed by several laboratories using steady-state fluorescent polarization, primarily represent differences due to the order component of membrane fluidity. That the rate or dynamic component may play some role in the differences was also suggested by the data obtained using the AS probes and a trend in rotational correlational times. These data are also consistent with cholesterol being primarily responsible for differences in BBM and BLM fluidity as the effect of cholesterol is on membrane order [27]. In addition, these data are in agreement with recently published time-resolved fluorescent anisotropy results from rabbit renal cortical BBM and BLM [30].

To determine whether a fluidity gradient existed within renal cortical brush border and basolateral membrane bilayers two different groups of fluorescent probes which imbed within the bilayer at different levels were used. DPH is a rod-shaped molecule that orients with high affinity in the hydrophobic regions (core) of the bilayer. TMA-DPH, because of the bulky charged trimethyl ammonium group, is restricted to a more superficial region of the bilayer. These probes, therefore, can be used to evaluate for differences between the inner core and superficial bilayer membrane fluidity. The AS probes can also be used to differentiate whether the bilayer has a fluidity gradient across it, as the anthroyloxy group can be positioned at different positions of the stearic acid moiety [12]. For our purposes, we chose the 2 and 12-AS probe because these would more closely evaluate the same regions of the bilayer as the DPH and TMA-DPH probes. Using both sets of probes, an apparent fluidity gradient was observed in both renal cortical brush border and basolateral membranes. For each membrane fraction the inner core portion of the bilayer was found to be more fluid than the superficial aspects of the bilayer. This is in agreement with the theoretic calculations regarding the movement of acyl chains as one moves farther from their point of attachment [17]. The difference noted for DPH and TMA-DPH, however, may not be as pronounced as it would seem due to a shorter lifetime for TMA-DPH [21]. Lifetimes were also not measured for the AS probes and as 2-AS may have a shorter lifetime than 12-AS [26], our gradient data is only suggestive. It was also noted, using both sets of probes, that the brush border membrane was less fluid at both inner core and superficial portions of the bilayer than was the basolateral membrane. This was true whether the determinations were made in intact membranes, total lipids, or phospholipid extracts from the two membrane fractions.

In summary, these studies indicate cholesterol is in large part responsible for the differences in membrane fluidity between BBM and BLM fractions, but that other factors also play a role. In addition, the differences measured between brush border and basolateral membrane fluidity are primarily due to alterations in the order component, but differences with respect to the rate or dynamic component of membrane fluidity may also be present.

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