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Biophysical and biochemical alterations of renal cortical membranes in diabetic rat

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The objective of this study was to determine whether streptozotocin-induced diabetes mellitus in the rat causes alterations in the lipid composition and fluidity of renal brush border membranes (BBM) and basolateral membranes (BLM). Compared to membranes of non-diabetic rats, BBM and BLM of diabetic rats contained 31% and 26%, respectively, less arachidonic acid and 36% and 46%, respectively, more linoleic acid esterfied in phospholipids. These changes were accompanied by a decrease in the average number of double bonds per mole of fatty acid, a measure of fatty acid unsaturation. In diabetic rats BLM had a higher total phospholipid/protein ratio (567 ± 20 vs. 482 ± 15 nmol/mg protein, $P < 0.01$), less cholesterol (369 ± 30 vs. 512 ± 34 nmol/mg protein, $P < 0.01$), more phosphatidylcholine (+72%) and less sphingomyelin (−22%) than did BBM. These differences were identical to those observed between BLM and BBM of non-diabetic rats. In control rats BLM was more fluid than BBM as assessed by the steady state fluorescence anisotrophy of diphenylhexatriene and by glycerol permeability. In diabetic rats the fluidity of BLM was not different from that of BBM as assessed by the steady state fluorescence anisotrophy of diphenylhexatriene whereas BLM was slightly more fluid than BBM as assessed by glycerol permeability. By both measures BLM and BBM from diabetic rats were significantly less fluid than BLM and BBM from control rats. Removal of proteins and cholesterol in sequence was accompanied by an increase in membrane fluidity in both groups. However, in no instance did the removal of proteins or cholesterol abolish the difference between the fluidity of diabetic membranes and that of control membranes. From these data we conclude that the reduction in fluidity of renal BLM and BBM in the diabetic rat is due to the change in the composition of fatty acids esterified in membrane phospholipids.

Introduction

The quantity of arachidonic acid esterified in phospholipid has been found to be depressed in a number of tissues of diabetic man and experimental animals [1–5]. We recently confirmed that arachidonic acid esterified in phospholipid is depressed in the renal cortex of rats with streptozotocin-induced diabetes mellitus [6]. The potential significance of this finding derives from the fact that the fatty acid composition of phospholipids plays a major role in determining the biophysical properties of a membrane. For example, the fluidity of a membrane is influenced by the length and the degree of unsaturation of the fatty acyl chains [7–9]. Other determinants of membrane fluidity include cholesterol content, lipid/protein ratio, and phosphatidylcholine/sphingomyelin ratio [10–12]. The goal of this study was to determine (1) whether the renal brush-border membrane (BBM) and basolateral membrane (BLM) of diabetic rats have altered fatty acid composition; (2) whether the biophysical properties of these membranes are altered; and (3) the contribution of the alteration in fatty acid composition to the alteration in biophysical properties. Thus, we analyzed first the lipid composition (fatty acids, phospholipids and cholesterol) of BBM and BLM isolated from the renal cortex of diabetic and non-diabetic rats and then evaluated the fluidity, as measured by fluorescence polarization, and glycerol permeability of these membranes and of liposomes made up of lipids extracted from these membranes. The results indicate that renal BBM and BLM obtained from diabetic rats are less fluid than those obtained from non-diabetic rats and that this change is due primarily to altered fatty acid composition of membrane phospholipids.

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Methods

Membrane preparation

Renal cortical plasma membranes were obtained from diabetic and age-matched non-diabetic male Sprague-Dawley rats. Diabetes mellitus was induced by a single intravenous injection of streptozotocin (65 mg/kg). Diabetic and age-matched control rats were allowed free access to food and water. 4 to 5 weeks after stable hyperglycemia was established, rats were killed by exsanguination from the aorta under pentobarbital anesthesia. The kidneys were removed and the cortex was dissected free. The cortices from 3 rats were pooled, minced with a razor blade and then homogenized. BBM and BLM were prepared using differential centrifugation and Percoll gradient techniques as previously reported from our laboratory [13]. The purity of membrane preparations was evaluated by measurement of the marker enzymes, alkaline phosphatase and Na^+/K^+-ATPase. The BBM fractions of control and diabetic rats were enriched in alkaline phosphatase activity 9.4 ± 0.5 and 9.1 ± 0.5-fold, respectively, compared to homogenate whereas Na^+/K^+-ATPase activity was enriched only 1.5 ± 0.3 and 1.4 ± 0.1-fold, respectively. The BLM fractions of control and DM rats were enriched in Na^+/K^+-ATPase activity 11.1 ± 1.0 and 11.2 ± 0.7-fold, respectively, whereas alkaline phosphatase activity was enriched 2.2 ± 0.3 and 2.3 ± 0.2-fold, respectively.

Extraction of membrane lipids

Total lipids were extracted from a quantity of membranes containing 0.4 to 0.6 mg protein. Membranes were pelleted at 4°C and the supernatant was removed. The pellet was resuspended in 1 ml of methanol containing 2% HCl and allowed to sit in ice for 5 min. Chloroform (1 ml) was then added to the suspension following which the suspension was stored under N_2 for 12 h at −20°C. Subsequently, an additional 1 ml of chloroform was added and the entire mixture was filtered through a Whatman GF/C filter to remove protein and other insoluble membrane components after which the filter was washed with 1 ml of chloroform/methanol (2:1, v/v)). The filtrate which contained the extracted lipids was dried under a stream of N_2 and the dry film was dissolved in a known volume of chloroform/methanol (2:1, v/v). All organic solvents contained 1% of 2,6-di-tert-butyl-4-methylphenol, an antioxidant.

Extraction of cholesterol

An aliquot of total lipid extract was brought to dryness and then re-dissolved in chloroform. The sample was applied to a silicic acid column, and the column was washed with 20 ml of chloroform which eluted cholesterol and other neutral lipids. Subsequently, phospholipids were eluted with 20 ml of methanol. In pilot experiments the recoveries of cholesterol and of phospholipid from the column were determined to be greater than 96%.

Preparation of liposomes

Multilamellar liposomes composed of total lipids or of extracted phospholipids were prepared by established methods [14]. Dried lipids were resuspended in 0.15 M KCl and 10 mM Tris (pH 7.0), and vortexed at room temperature after which they were stored under N_2 at room temperature overnight before use.

Lipid analysis

Total phospholipids and individual phospholipids were quantitated by measuring inorganic phosphorus (P_i) content as previously reported from our laboratory [13]. Individual phospholipids were separated by two-dimensional thin-layer chromatography using chloroform/methanol/28% aqueous ammonia (65:35:5, v/v) and chloroform/acetone/methanol/acetic acid/water (10:4:2:2:2, v/v) [15]. Spots were visualized by exposure to iodine vapor and identified by simultaneous thin layer chromatography of authentic standards. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin were clearly separated. Spots were scraped off the plate and analyzed for P_i. Fatty acids were analyzed by gas liquid chromatography as previously reported from our laboratory [16]. An aliquot of total lipid extract was dried under N_2, after which it was redissolved in benzene in a test tube. Sodium methoxide (0.5 M) in anhydrous methanol (2 ml) was added. Then the mixture was maintained at 50°C for 40 min to ensure complete transesterification of fatty acids. Glacial acetic acid (0.1 ml) was added followed by water (5 ml) followed by hexane (5 ml × 2) to extract the fatty acid methyl esters which were subsequently analyzed on a Perkin Elmer gas liquid chromatograph (model 8500) equipped with a steel column of SP 2340 resin. Injector temperature was 200°C. Oven temperature was programmed between 100–180°C over 35 min. Using a phospholipid mixture of known compositon we determined that this method yields fatty acid recoveries greater than 95%. Cholesterol was quantitated by the *o*-phthalaldehyde method [17].

Fluorescence polarization

1,6-Diphenyl-1,3,5-hexatriene was used as the fluorescent probe [10]. BBM and BLM vesicles (200–250 μg protein) were incubated with 1 μM diphenylhexatriene in 0.1 M phosphate buffer (pH 7.4). The suspension was incubated at 37°C in a shaking H_2O bath for 1 h and kept in the dark at room temperature for 1–2 h. Fluorescence intensities were measured in a Perkin Elmer LS5 spectrofluorometer. The sample chamber

TABLE I

Fatty acid composition of phospholipid in renal cortical brush-border membranes and basolateral membranes from control and diabetic rats

Data represent means ± S.E. in units of mole for eight experiments. Numbers in parenthesis represent number of carbons followed by number of double bonds per moledule of fatty acid. Δ/mol equals average number of double bonds per mol fatty acid.

	Brush-border membrane			Basolateral membrane		
	control	diabetic	*P*	control	diabetic	*P*
Hexadecanoic (palmitic) (16:0)	15.1 ±0.4	17.3 ±0.3	< 0.01	17.4 ±0.2	18.3 ±0.3	< 0.0
Hexadecenoic (palmitoleic) (16:1)	1.0 ±0.2	1.2 ±0.2	N.S.	1.0 ±0.1	0.8 ±0.1	N.S.
Octadecanoic (stearic) (18:0)	20.4 ±0.4	22.4 ±0.4	< 0.01	21.6 ±0.3	21.7 ±0.5	N.S.
Octadecanoic (oleic) (18:1)	10.7 ±0.4	12.6 ±0.2	< 0.01	12.9 ±0.3	13.9 ±0.05	< 0.0
Octadecadienoic (linoleic) (18:2)	13.8 ±0.5	18.7 ±0.3	< 0.01	12.3 ±0.5	18.0 ±1.0	< 0.0
Octadecatrienoic (linolenic) (18:3)	1.5 ±0.5	0.9 ±0.3	N.S.	1.3 ±0.3	1.3 ±0.2	N.S.
Eicosatetraenoic (arachidonic) (20:4)	31.6 ±0.7	21.8 ±1.1	< 0.01	29.9 ±0.9	21.6 ±1.0	< 0.0
Docosanoic (behenic) (22:0)	2.4 ±0.4	1.9 ±0.2	N.S.	1.4 ±0.2	1.6 ±0.3	N.S.
Docosapentaenoic (22:5)	0.4 ±0.1	0.4 ±0.2	N.S.	0.4 ±0.1	0.3 ±0.1	N.S.
Docosahexaenoic (22:6)	1.7 ±0.3	1.3 ±0.5	N.S.	1.2 ±0.3	1.7 ±0.4	N.S.
Δ/mol	1.84±0.01	1.52±0.02	< 0.01	1.70±0.02	1.53±0.02	< 0.0

temperature was controlled by a circulating water bath and sample temperature was monitored by a thermometer equipped with a thermocouple. The excitation and emission wavelengths were 360 nm and 410 nm, respectively. Fluorescence intensity was measured perpendicular ($I_\perp$) and parallel ($I_\parallel$) to the polarization plane of the exciting light. Steady state fluorescence anisotropy ($\bar{r}_s$) was calculated according to the equation $\bar{r}_s = (I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)$ [10]. Measurements were made at 25, 30 and 37°C. Fluorescence anisotropy of liposomes was measured as described for BBM and BLM. An aliquot of total lipid or phospholipid extract was dried, redissolved in a 0.1 M phosphate buffer and incubated with diphenylhexatriene as described above.

Glycerol permeability

Membrane vesicles or multilamellar liposomes were suspended in 0.15 M KCl in 0.1 M Hepes buffer at pH 7.4. Aliquots of 40 μl were added to 1 ml of 0.3 M glycerol and absorbance was measured at 450 nm and 37°C as a function of time as previously reported from our laboratory [18].

Statistical significance of differences within the control or experimental groups was assessed by analysis of variance and the Duncan new multiple range rest. Statistical significance of differences between control and experimental groups was assessed by Student's *t*-test.

Materials

Phospholipid standards were purchased from Avanti Polar Lipids (Birmingham, AL); the fatty acid standards and silicic acid column were purchased from Supelco (Supelco Park, Bellefonte, PA); silica gel G-60 thin layer chromatography plates were purchased from Analtech (Neward, DE); the steel column prepacked with SP2340 resin was purchased from Perkin Elmer (Norwalk, CT); all other chemicals and reagents were purchased from Sigma Chemicals (St. Louis, MO).

Results

Fatty acid composition

The composition of fatty acids esterified in phospholipids of BBM and BLM from the renal cortex of control and diabetic rats is summarized in Table I. Within each group, the fatty acid compositions of BBM and BLM were similar. Arachidonic acid (20:4) was

TABLE II

Phospholipid composition of renal cortical brush-border membranes and basolateral membranes from control and diabetic rats

Data represent means ± S.E. in units of nmol/mg protein for eight experiments. There were no significant differences between the phospholipid composition of diabetic and control membranes.

	Brush-border membrane		Basolateral membrane	
	control	diabetic	control	diabetic
Phosphatidylcholine	104.7±7.3	103.1 ±9.5	174.7±8.4	177.1±3.1
Phosphatidylethanolamine	120.8±5.8	119.3 ±8.4	174.9±5.0	178.8±8.9
Sphingomyelin	128.5±9.1	119.9 ±7.9	95.1±8.0	93.2±5.3
Phosphatidylserine	58.9±3.8	51.7 ±3.6	55.7±6.8	59.2±7.2
Phosphatidylinositol	25.2±5.7	25.01±5.1	24.6±2.8	34.7±4.6

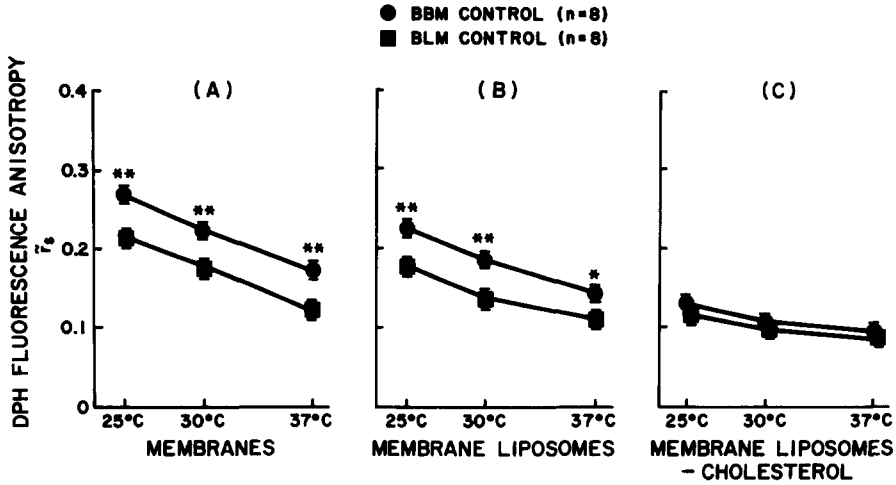

Fig. 1. Comparison of diphenylhexatriene (DPH) fluorescence anisotropies ($\bar{r}_s$) of control renal brush border membrane (BBM) and basolateral membrane (BLM) as a function of temperature. Symbols represent means ± S.E. **,*, significantly different from BLM, $P < 0.01$ and $P < 0.05$, respectively.

significantly depressed in renal BBM and BLM of diabetic rats compared to the arachidonic acid content of renal BBM and BLM of control rats. The reduction of arachidonic acid was accompanied by significant increases of linoleic acid (18:2), oleic acid (18:1) and palmitic acid (16:0) in both plasma membranes. This shift towards more saturated fatty acids was reflected by a decline in the average number of double bonds per mole of fatty acid (Δ/mol) from 1.84 ± 0.01 to 1.52 ± 0.02 ($P < 0.01$) in the case of BBM and from 1.70 ± 0.02 to 1.53 ± 0.02 ($P < 0.01$) in the case of BLM (Table I).

Phospholipid composition

No differences were observed between the total phospholipid of diabetic BBM and control BBM (482 ± 15 vs. 477 ± 22 nmol/mg protein) or between the total phospholipid of diabetic BLM and control BLM (567 ± 20 vs. 560 ± 22 nmol/mg protein). BLM of both diabetic and control rats contained significantly more phospholipid per mg membrane protein than BBM, ($P < 0.01$). No significant differences were found between the phospholipid composition of diabetic and control BBM or between the phospholipid composition of diabetic and control BLM (Table II). BLM of control and diabetic rats contained 67% and 72% more phosphatidylcholine and 26% and 22% less sphingohyelin, respectively than did BBM. Thus, the phosphatidylcholine/sphingomyelin ratios of control BLM (1.93 ± 0.18) and diabetic BLM (1.93 ± 0.09) were significantly higher than the ratios of control BBM (0.86 ± 0.10) and diabetic BBM (0.88 ± 0.09), $P < 0.01$. In both groups phosphatidylethanolamine was also higher in BLM compared to BBM.

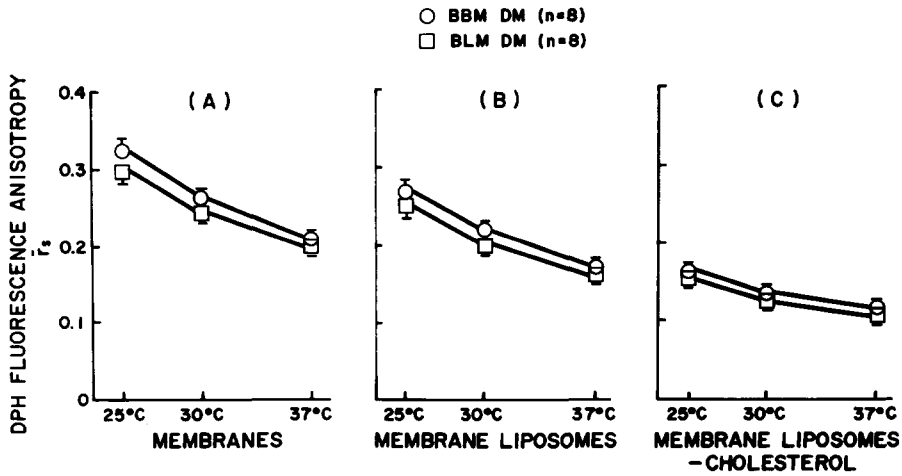

Fig. 2. Comparison of diphenylhexatriene (DPH) fluorescence anisotropies ($\bar{r}_s$) of renal brush-border membrane (BBM) and basolateral membrane (BLM) from rats with diabetes mellitus (DM) as a function of temperature. Symbols represent means ± S.E.

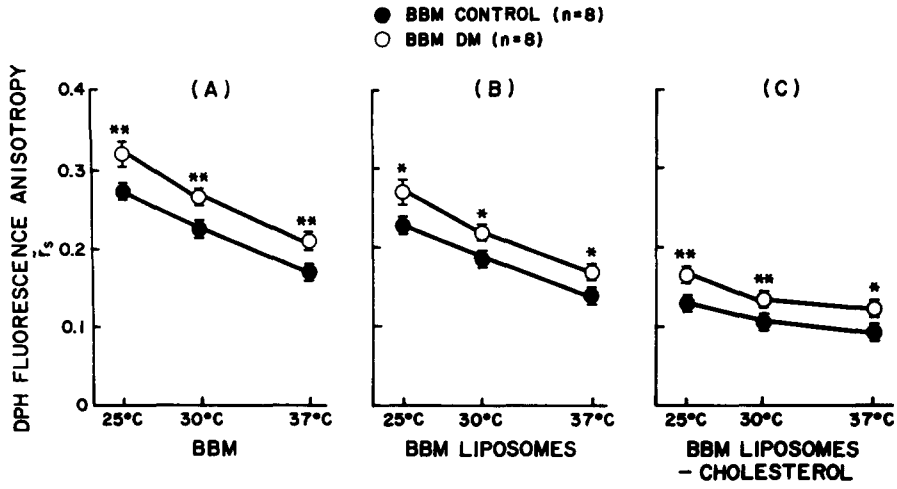

Fig. 3. Comparison of diphenylhexatriene (DPH) fluorescence anisotropies ($\tilde{r}_s$) of renal brush-border membrane (BBM) from control rats and rats with diabetes mellitus (DM) as a function of temperature. Symbols represent means ± S.E. **,*, significantly different from control, $P < 0.01$ and $P < 0.05$, respectively.

Cholesterol composition

Control BBM contained 44% more cholesterol than control BLM (507 ± 27 vs. 351 ± 15 nmol/mg protein). The quantities of cholesterol found in BBM and BLM of diabetic rats (512 ± 34 vs. 369 ± 30 nmol/mg protein) were not different from those of control. The total phospholipid/cholesterol mole ratio was significantly higher in BLM compared to BBM in control (1.61 ± 0.08 vs. 0.97 ± 0.08, $P < 0.01$) and in diabetic rats (1.63 ± 0.16 vs. 0.88 ± 0.07, $P < 0.01$).

In summary, BLM was similar to BBM with respect to fatty acid composition; however, BLM contained more total phospholipid, phosphatidylcholine and phosphatidylethanolamine but less sphingomyelin and cholesterol than did BBM. This was true for both control and diabetic rats. The only significant difference between the lipid composition of control and diabetic membranes was an alteration of the fatty acid profile with the most prominent change being a marked reduction of arachidonic acid in both BBM and BLM of diabetic rats.

Biophysical properties

The steady state fluorescence anisotrophy ($\tilde{r}_s$) of diphenylhexatriene in control BBM was significantly greater than that in control BLM. (Fig. 1A). Removal of membrane protein resulted in equivalent declines of $\tilde{r}_s$ in liposomes prepared from control BBM and control BLM so that the significant difference between the $\tilde{r}_s$ of BBM and BLM liposomes persisted (Fig. 1B). The sequential removal of cholesterol eliminated the difference between the $\tilde{r}_s$ of BBM liposomes and that of

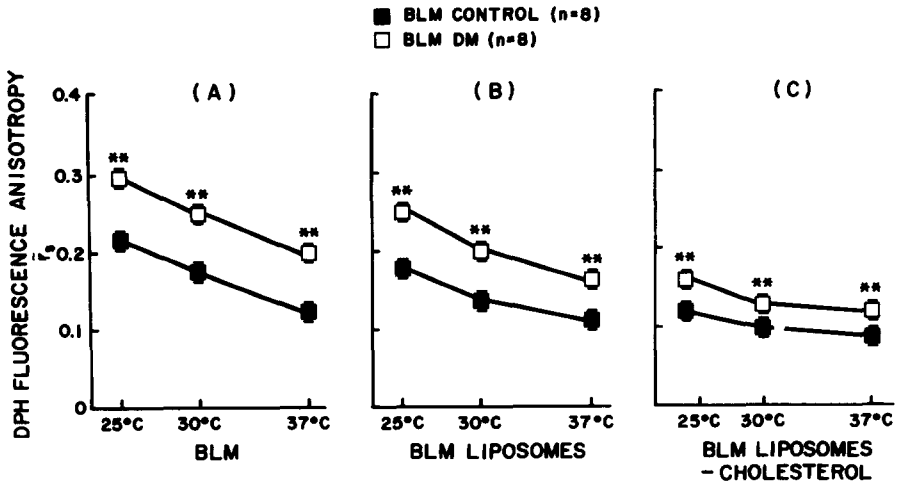

Fig. 4. Comparison of diphenylhexatriene (DPH) fluorescence anisotropies ($\tilde{r}_s$) of renal basolateral membrane (BLM) from control rats and rats with diabetes mellitus (DM) as a function of temperature. Symbols represent means ± S.E. **,*, significantly different from control, $P < 0.01$ and $P < 0.05$, respectively.

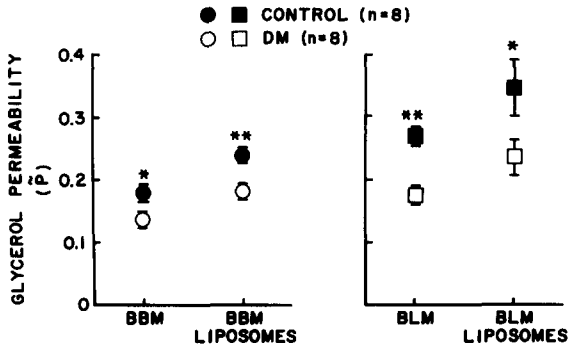

Fig. 5. Comparison of glycerol permeabilities ($\tilde{P}$) of renal brush-border membrane (BBM) and basolateral membrane (BLM) from control rats and rats with diabetes mellitus (DM). Symbols represent means ± S.E. **·*, significantly different from DM, $P < 0.01$ and $P < 0.05$, respectively.

BLM liposomes (Fig. 1C). In contrast, no significant differences were observed between the $\tilde{r}_s$ of diabetic BBM and BLM (Fig. 2A) or of membranes devoid of proteins (Fig. 2B) and cholesterol (Fig. 2C). The $\tilde{r}_s$ of BBM from diabetic rats was significantly greater than that of control BBM (Fig. 3A) and this difference persisted following the removal of membrane proteins (Fig. 3B) and cholesterol (Fig. 3C). The $\tilde{r}_s$ of BLM from diabetic rats was also significantly greater than that of control BLM (Fig. 4A); and, similar to the pattern observed for BBM, this difference persisted after the removal of membrane proteins (Fig. 4B). Following removal of cholesterol the difference between the $\tilde{r}_s$ of DM and control liposomes became less pronounced, but it remained significant (Fig. 4C).

In agreement with the diphenylhexetriene fluorescence anisotropy data, the permeability of control BLM to glycerol was significantly greater than that of control BBM (0.265 ± 0.019 vs. 0.181 ± 0.011, $P < 0.01$). A significant difference was also detected between the glycerol permeabilities of diabetic BLM and BBM (0.174 ± 0.010 vs. 0.136 ± 0.038, $P < 0.05$). Control BLM was more permeable to glycerol than was diabetic BLM and control BBM was more permeable to glycerol than was DM BBM (Fig. 5). Removal of protein resulted in a similar rise in glycerol permeability of all liposomes so that the difference between control and diabetic membranes persisted (Fig. 5).

Discussion

The results of our experiments provide the first documentation that the quantity of arachidonic acid esterified in phospholipid is greatly depressed in renal cortical BBM and BLM of rats with streptozotocin-induced diabetes mellitus. The decrease of arachidonic acid was associated with a shift towards more saturated fatty acids as reflected by the decline in the average number of double bonds per mole of esterified fatty acid. Because the degree of fatty acyl chain unsaturation has been shown to be a determinant of membrane fluidity [7–9], we sought to assess to what extent the fluidity of renal cortical membranes was altered in diabetic rats.

For this purpose we measured the steady state fluorescence anisotrophy ($\tilde{r}_s$) of diphenylhexatriene, a probe that has been used extensively to assess the influence of sterols, phospholipid head groups and phospholipid acyl chains on membrane fluidity [9–12]. Anisotrophy values vary inversely with membrane fluidity. In our experiments $\tilde{r}_s$ of diphenylhexatriene was signicantly higher in BBM than in BLM of non-diabetic rats. This observation confirms the findings of previous investigators [19–22] that renal cortical BBM is less fluid than renal cortical BLM. In contrast no difference was detected between $\tilde{r}_s$ of diphenylhexatriene in BBM and that in BLM of diabetic rats. Furthermore, $\tilde{r}_s$ in diabetic BBM was significantly higher than that in control BBM and $\tilde{r}_s$ in diabetic BLM was significantly higher than that in control BLM. These data support the conclusion that in the diabetic rat the fluidity of renal cortical BBM is similar to that of renal cortical BLM and that both membranes are significantly less fluid than renal cortical BBM and BLM of the non-diabetic rat.

These conclusions rest on the assumption that the fluorescence lifetimes of the probe in BBM and BLM from diabetic and non-diabetic rats are similar. Although we did not measure this variable in our experiments, other investigators have detected no difference between the fluorescence lifetimes of diphenylhexatriene measured in renal cortical BBM and BLM from non-diabetic rats [19–23]. To our knowledge similar measurements have not been performed using renal cortical membranes of the diabetic rat. However, Dudeja et al. [24] have reported no difference between the fluorescence lifetimes of diphenylhexatriene in small intestinal BBM from control and diabetic rats. Based on these data it appears reasonable to assume that the fluorescence liftimes of diphenylhexatriene in renal cortical membranes from diabetic rats are the same as that in renal cortical membranes from non-diabetic rats.

Glycerol permeability has been shown to vary directly with membrane fluidity [14,25,26]. Therefore, this technique was used as an independent method for corroborating the results obtained with diphenylhexatriene fluorescence polarization. The $\tilde{r}_s$ data predicted that the glycerol permeabilities of BBM and BLM from diabetic rats would be significantly lower than those of BBM and BLM from control rats. This prediction was confirmed (Fig. 5). The $\tilde{r}_s$ data also predicted that the glycerol permeability of control BLM would be higher than that of control BBM. This prediction was also confirmed (Fig. 5). Finally the $\tilde{r}_s$ data predicted that

the glycerol permeabilities of BBM and BLM would be the same. This prediction was not confirmed. We found that the glycerol permeability of diabetic BLM was slightly but significantly greater than that of diabetic BBM. It should be noted, however, that the $\tilde{r}_s$ of diabetic BLM tended to be lower than that of diabetic BBM so that the deviation with respect to membrane fluidity was in the expected direction. Furthermore, since glycerol permeability provides a composite measure of the barrier function of the entire membrane, whereas diphenylhexatriene fluorescence polarization is thought to assess the degree of order at the level of the hydrocarbon chains [10], it is likely that the minor discrepancy in these results reflects a perturbation in a region of the membrane (i.e., the hydrogen belt) that influences glycerol permeability but not diphenylhexatriene fluorescence polarization.

We next sought to ascertain the major factor(s) responsible for the reduced fluidity of BBM and BLM of diabetic rats. Membrane fluidity can be altered by a quantitative or qualitative change in membrane proteins [10]. However, neither factor could be implicated in our study. The phospholipid/protein ratios of BBM and BLM were the same as those of control membranes; and, in agreement with previous reports [19,23] we found that the phospholipid/protein ratio of BLM was significantly higher than that of BBM. Although removal of membrane proteins resulted in a significant fall in $\tilde{r}_s$ and a significant rise in glycerol permeability in all membranes, in no instance did removal of proteins eliminate or even significantly reduce the differences between the diphenylhexatriene fluorescence anisotropies or the glycerol permeabilities of membranes from control and diabetic rats.

The phospholipid composition of membranes has also been shown to influence membrane fluidity [10–12]. In the case of renal BBM and BLM the phosphatidylcholine/sphingomyelin ratio has been implicated as an important determinant of membrane fluidity [27]. We confirmed the previously reported observation in non-diabetic rats that BBM contains less phosphatidylcholine and more sphingomyelin than does BLM [19,23]. A similar difference between the phospholipid composition of BBM and BLM was observed in diabetic rats. In fact the phosphatidylcholine/sphingomyelin ratios of BBM and BLM from diabetic rats were identical to those of control BBM and BLM, respectively. Clearly some factor other than the phosphatidylcholine/sphingomyelin ratio is responsible for the decreased membrane fluidity in diabetic rats.

Cholesterol has also been shown to be an important determinant of membrane fluidity [10,12,22]. We confirmed that BBM has a higher cholesterol content than does BLM in control rats [23], and that the higher cholesterol content is the major determinant of the difference between the $\tilde{r}_s$ of diphenylhexatriene in BBM and BLM in these rats [22]. This was shown by the equalization of $\tilde{r}_s$ after the extraction of cholesterol from liposomes. The cholesterol content of diabetic membranes was identical to that of control membranes. However, in contrast to control membranes, the difference between the cholesterol contents of BBM and BLM in diabetic rats was not manifested by a difference in membrane fluidity as assessed by the $\tilde{r}_s$ of diphenylhexatrine. Although $\tilde{r}_s$ declined significantly in response to the removal of cholesterol from liposomes prepared from diabetic membranes, it remained significantly higher than that of control liposomes devoid of cholesterol.

Thus, the decreased fluidity of diabetic membranes compared to control membranes cannot be explained by differences in the ratio of total phospholipid/protein, by a qualitative difference in membrane protein, by differences in the composition of membrane phospholipid, by differences in the ratio of phosphatidylcholine/sphingomyelin or by differences in membrane cholesterol content. By a process of elimination we conclude that the decreased fluidity of diabetic membranes is due to the change in the composition of fatty acids esterified in membrane phospholipid.

One mechanism by which a shift towards less saturated fatty acids may influence membrane fluidity is the associated change in the effective hydrophobic length of the fatty acyl chain. The effective hydrophobic length is thought to be an important determinant of the hydrophobic interaction between acyl chains, i.e., the 'tightness' of packing of the chains in the hydrophobic region of the membrane [28]. Increased packing is associated with increased membrane viscosity or decreased membrane fluidity. The effective hydrophobic length of a fatty acid is equal to the total number of carbon atoms in the chain minus the carbon and its neighbor involved in ester linkage minus those carbons which participate in double bonds [28]. For example the effective hydrophobic length of palmitic acid (16:0) is 14, whereas that of arachidonic acid (20:4) is only 10. From the data in Table I we calculated the mean effective hydrophobic length of the fatty acyl chains of control BBM to be 12.55 ± 0.05 whereas the mean effective hydrophobic length of the fatty acyl chains of diabetic BBM was 13.00 ± 0.05 ($P < 0.01$). In control BLM the mean effective hydrophobic length was 12.77 ± 0.05 whereas it increased to 12.99 ± 0.04 in diabetic BLM ($P < 0.01$). The differences between the effective hydrophobic length of fatty acyl chains in diabetic and control membranes, although quantitatively small, are highly significant, statistically. Moreover, other investigators have demonstrated that small changes in the effective hydrophobic lengths of fatty acyl chains in liposomes induce large changes in membrane fluidity and glycerol permeability

[25]. Thus, it is possible that the slight increase in effective hydrophobic length detected in our experiments could explain, at least in part, the observed decrease in membrane fluidity of renal BBM and BLM in diabetic rats.

It should be emphasized that the changes in the biophysical properties of renal BBM and BLM in the diabetic rat are also likely to occur in other organs and tissues of the diabetic animal that manifest alteration in the composition of fatty acids esterified in phospholipid. For example, we recently demonstrated that RBC membranes of rats with streptozotocin-induced diabetes mellitus have decreased arachidonic acid content and exhibit decreased membrane fluidity as assessed by diphenylhexatriene fluorescence anisotropy and decreased deformability as assessed by a filtration technique [29]. Similar changes have been observed in RBC membrane of humans with diabetes mellitus and the changes are reversed by insulin [30]. Since membrane fluidity has been shown to influence a number of membrane-linked functions including transport processes, signal transduction and enzyme activities [9,12,20,31–33], the possibility exists that a generalized alteration of this fundamental property of membranes may contribute to the metabolic, functional and structural complications of diabetes mellitus.

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