

Lipid Composition and Fluidity in the Jejunal Brush-Border Membrane of Spontaneously Hypertensive Rats. Effects on Activities of Membrane-Bound Proteins

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The lipid composition and fluidity of jejunal brush-border membrane vesicles (BBMV) have been studied in spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto (WKY) rats. The activities of both Na⁺-dependent D-glucose cotransport and Na⁺-H⁺ antiport have also been determined. A significant increase in the level of free cholesterol was observed in jejunal BBMV from SHR compared to WKY rats. Since phospholipid values did not change in either group of animals, a significant enhancement in the free cholesterol/phospholipid ratio was observed in SHR. A decrease in the levels of phosphatidylethanolamine together with an increase in the values of phosphatidylserine was observed in hypertensive rats. Although the content of phosphatidylcholine (PC) and sphingomyelin (SM) was not significantly altered in SHR, the ratio PC/SM significantly increased in these animals when compared to WKY rats. The major fatty acids present in brush-border membranes prepared from SHR and WKY rats were palmitic (16:0), stearic (18:0), oleic (18:1, n-9) and linoleic (18:2, n-6), and the fatty acid composition was not modified by the hypertension. A decreased fluorescence polarization, i.e., increased membrane fluidity, was observed in SHR, which was not correlated to the increased ratio of cholesterol/phospholipid found in the brush-border membrane isolated from these animals. These structural changes found in SHR were associated to an enhancement in both Na⁺-dependent D-glucose transport and Na⁺-H⁺ antiport activity in the jejunal BBMV of SHR.

KEY WORDS: Spontaneously hypertensive rat; brush-border membrane; lipid fluidity; lipid composition; D-glucose; Na⁺-H⁺ exchanger.

ABBREVIATIONS: BBMV, brush-border membrane vesicles; DPH, 1,6-diphenyl-1,3,5-hexatriene; FC, free cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; SHR, spontaneously hypertensive rat; p, steady-state fluorescence polarization; r_s, steady-state fluorescence anisotropy; WKY, Wistar Kyoto.

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INTRODUCTION

Hypertension has been associated to multiple abnormalities in cell membrane functions (1,2), including the mobilization of intracellular calcium stores (3), changes in Na^+ - K^+ cotransport, Na^+ - Li^+ countertransport and Na^+ - H^+ exchanger (4,5).

It has been suggested that a generalized physico-chemical alteration of the lipid bilayer, the matrix in which all the membrane proteins function, may be responsible for these multiple membrane abnormalities (6,7,8). Thus, previous reports have found consistent differences in the lipid composition of erythrocyte and platelet membranes and other tissues in hypertension (9,10,11).

In addition, changes in the lipid composition of erythrocyte and intestinal brush-border membranes by dietary lipid modifications (12,13) have been shown to alter the physical properties of the membrane, resulting in modifications of the lipid-protein interaction which in turn might influence the function of a wide variety of intrinsic and extrinsic membrane-bound proteins.

In this study we explored the lipid composition and fluidity of jejunal brush-border membranes from spontaneously hypertensive (SHR) and normotensive-control (WKY) rats. Taking into account the association between such parameters and the function of membrane-bound proteins, we also examined the activity of two membrane-bound proteins such as Na^+ -dependent D-glucose transporter and Na^+ - H^+ exchanger.

The results described below demonstrate that jejunal brush-border membranes from SHR, when compared to those from WKY rats, had increased the cholesterol-phospholipid ratio, the lipid fluidity and, concomitantly, both Na^+ -dependent D-glucose transport and Na^+ - H^+ antiport activity.

MATERIALS AND METHODS

Materials

D-(U- ^{14}C) Glucose (283 mCi/mmol) was purchased from Amersham International. Sodium 22 as sodium chloride (253 mCi/mg) was purchased from Dupont NEN. Membrane filters (Millipore GSWP02500) were obtained from Millipore Products Division, U.K. All other chemicals were supplied by Sigma.

Animal Model

Male SHR and WKY rats were obtained at the age of 8–9 weeks from IFFA CREDO (France). Animals had free access to food and water. All experiments were performed on 10–12 week-old-rats, which were fasted during 18 h before sacrifice.

Brush-Border Membrane Vesicles Preparation

Brush-border membrane vesicles (BBMV) were isolated from the jejunal mucosa by the $MgCl_2$ precipitation method (14) with slight modifications. The mucosal scrapings were homogenized for 3 min in 100 mM mannitol, 2 mM HEPES/Tris, pH 7.1, using a Waring blender at maximal speed. The homogenate was treated with 10 mM $MgCl_2$ and centrifuged at 3000 g for 30 min. The following steps were taken as previously described (14). All isolation steps were carried out on ice. Aliquots of BBMV were stored under liquid nitrogen until analysis. Isolated BBMV were checked by determination of the specific activity of the brush-border membrane marker enzyme sucrase with the method of Dahlquist (15). The final brush-border membrane fraction showed a 18–21 fold enrichment in sucrase activity with respect to the original homogenate in both groups of animals. Cross contamination with the basolateral membrane was negligible, as there was no enrichment of Na^+K^+ -ATPase (16). Protein was measured by a Coomassie Brilliant Blue protein assay using bovine gamma-globulin as standard (17).

Lipid Analysis

Total lipids were extracted from the brush-border membrane by the method of Folch (18) using chloroform/methanol (2:1, v/v) as the solvent system and 0.02% (w/v) butylated hydroxytoluene as antioxidant. Lipid and phospholipid compositions were obtained by means of the Iatroscan TLC/FID technique (19). The Iatroscan MK-5 was used in combination with Chromarods S, having a precoated active silica thin layer. Chromarods S were routinely stored in 4.5 M H_2SO_4 . Prior to use, the rods were washed with distilled water, dried at 100°C for 15 min and activated by being passed through the flame of the Iatroscan detector. Three μ l of total lipids or phospholipids was spotted on each rod using a 10 μ l Hamilton syringe. To separate total lipids, rods were developed in hexane/diethyl ether/acetic acid (80:20:1, v/v/v). The phospholipids were resolved in two steps, starting with an initial development of rods in chloroform/methanol/acetic acid/water (201:84:9:6, v/v/v/v), drying at 70°C for 10 min, and a second development in hexane/diethyl ether/acetic acid (80:20:1, v/v/v). Rods were scanned under the following conditions: hydrogen flow, 150 ml/min; air flow, 1750 ml/min; scanning speed, 47 mm/s; chart speed, 42 mm/min. A Iatroscorder TC-11 integrator was used for recording and area integration.

Fatty Acid Analysis

Fatty acids of the total lipid extract were analyzed by gas chromatography (GC). The samples were saponified by heating for 5 min with 5 ml of 0.2 M sodium methylate and heated again at 80°C for 5 min with 6% (w/v) H_2SO_4 in anhydrous methanol. The fatty acid methyl esters thus formed were eluted with hexane, and analyzed in a Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detector and using an Omegawax 320 fused

silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film). The initial column temperature was 200°C, which was maintained for 10 min, then programmed from 200–230°C at 2°C/min. The injection and detector temperatures were 250°C and 260°C, respectively. The flow rate of helium was 2 ml/min, the column head pressure was 250 kPa and the detector auxiliary flow rate was 25 ml/min. Peak areas were calculated by a Hewlett-Packard 3990A recording integrator. Individual fatty acid methyl esters were identified on isothermal runs by comparison of their retention time against those of standards. Fatty acid methyl esters were quantified by internal standardization (tricosanoic methyl ester, 23:0), using peak-area integration.

Fluorescence Measurement

The steady-state fluorescence polarization (p) and fluorescence anisotropy (r_s) were determined as previously described (20), using the lipid-soluble fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). A stock solution of 2 mM DPH in tetrahydrofuran was prepared and stored in the dark at -30°C. BBMV equivalent to 100 μg protein were incubated at 25°C for 1 h in 2 ml of buffered saline containing 250 mM sucrose, 10 mM Tris-HCl, pH 7.4 and 2 μM DPH. Measurements were taken using a Perkin-Elmer 650-40 fluorescence spectrophotometer equipped with a polarizing filter. The excitation and emission wavelengths were 365 and 430 nm, respectively. The steady-state fluorescence polarization (p) was calculated as

$$p = (I_{vv} - I_{vh}) / (I_{vv} + I_{vh}),$$

where I_{vv} and I_{vh} are observed intensities measured with polarizers parallel to and perpendicular to, respectively, the vertically oriented polarizer exciting beam. The steady-state fluorescence anisotropy (r_s) was calculated from the ratio

$$r_s = 2p / (3 - p)$$

where p is the fluorescence polarization.

Corrections for light scattering were routinely made by subtracting the signal obtained from identical but unlabelled samples: this contributed less than 3% to the fluorescence of the complete system.

The vesicles were used for fluorescence polarization studies immediately after preparation.

Uptake Studies

The uptake of D-glucose was measured at 25°C by a rapid filtration technique. BBMV were suspended in a medium containing 300 mM mannitol, 0.1 mM MgSO₄, 0.02% (w/v) NaN₃ and 20 mM HEPES/Tris, pH 7.4. The assay was initiated by the addition of 100 μl of the incubation medium to a suspension of BBMV (75-125 μg of protein). The incubation medium contained 100 mM mannitol, 0.1 mM MgSO₄, 0.02% (w/v) NaN₃, 20 mM HEPES/Tris, pH 7.4, 0.1 mM ¹⁴C-D-glucose and either 100 mM NaSCN or 100 mM KSCN. The uptake

was terminated by the addition of an ice-cold stop solution. The stop solution contained 150 mM KSCN, 0.1 mM MgSO₄, 0.02% (w/v) NaN₃, 0.25 mM phlorizin, and 20 mM HEPES/Tris, pH 7.4. The BBMVs were separated from the incubation medium by filtering on a 0.22 μ m pore size Millipore filter. The filter was washed with 5 ml of stop solution. The radioactivity retained on the filter was measured using a scintillation counter. The uptake at time zero was measured and subtracted from the total radioactivity of each sample. All experiments were performed in duplicate.

For the Na⁺ uptake, BBMVs were suspended in a medium containing 300 mM mannitol, 50 mM potassium gluconate and 20 mM MES/Tris, pH 5.5. The assay was initiated by the addition of 100 μ l of the incubation medium to 8–10 μ l of a suspension of BBMVs (equivalent to 100–150 μ g of protein). The uptake was quenched by the addition of 1 ml of an ice-cold stop solution. The incubation medium contained 300 mM mannitol, 50 mM potassium gluconate, 0.1 mM sodium gluconate, 45 μ M valinomycin, tracers of ²²Na⁺ and either 20 mM MES/Tris (pH 5.5) or 20 mM HEPES/Tris (pH 7.5). The stop solution contained 300 mM mannitol, 50 mM potassium gluconate and 20 mM MES/Tris (pH 5.5).

Statistical Treatment of the Results

Results are expressed as the mean \pm SE. The statistical significance of the differences between groups was determined by the Student's t-test. Differences were considered significant when $p < 0.05$.

RESULTS

Lipid Composition

Brush-border membrane lipid composition of SHR and WKY rats are shown in Table 1. Free fatty acid levels were similar for both SHR and WKY rats. However, a significant increase in the level of free cholesterol was observed in SHR compared to WKY rats. Since phospholipid values did not change in either

Table 1. Lipid composition of jejunal BBMVs prepared from SHR and WKY rats

Lipid	Percentage (w/w) of total lipid	
	WKY	SHR
Phospholipids	64 \pm 4.6	58 \pm 2.6
Free fatty acids	29 \pm 4.3	32 \pm 3.0
Free cholesterol	9.8 \pm 0.8	20 \pm 2.7**
Free cholesterol/Phospholipid	0.2 \pm 0.02	0.5 \pm 0.12*

Results are means \pm SE for at least eight different preparations.

* $p < 0.05$.

** $p < 0.025$ compared to WKY rats.

Table 2. Phospholipid composition of jejunal BBMV prepared from SHR and WKY rats

Phospholipid	Percentage (w/w) of total phospholipid	
	WKY	SHR
Phosphatidylethanolamine	48 ± 5.7	35 ± 1.5**
Phosphatidylserine	34 ± 6.3	50 ± 3.1**
Phosphatidylcholine	14 ± 3.1	10 ± 1.2
Sphingomyelin	4.1 ± 0.5	3.2 ± 0.3
Phosphatidylinositol	<1	<1
Phosphatidylcholine/Sphingomyelin	2.1 ± 0.3	3.7 ± 0.5*

Results are means ± SE for at least eight different preparations.

* $p < 0.05$,

** $p < 0.025$ compared to WKY rats.

group of animals, a significant enhancement in the free cholesterol/phospholipid ratio was observed in the hypertensive rats. Phospholipid composition of jejunal BBMV for SHR and WKY rats is shown in Table 2. Respective phosphatidylethanolamine (PE) and phosphatidylserine (PS) levels were significantly decreased and increased in SHR compared to WKY rats. Although the content of phosphatidylcholine (PC) and sphingomyelin (SM) was not significantly altered by hypertension, the PC/SM ratio was significantly increased in SHR. No significant differences were noted in the total fatty acid composition of jejunal BBMV prepared from either SHR or WKY rats (Table 3). As shown in this

Table 3. Fatty acid composition of jejunal BBMV prepared from SHR and WKY rats

Component	Composition (% w/w)	
	WKY	SHR
Fatty acid		
14:0	1.5 ± 0.33	2.5 ± 0.6
16:0	21.4 ± 3.7	26.8 ± 0.9
16:1(n-7)	5.3 ± 1	5.2 ± 1.2
18:0	14.4 ± 1.7	13.6 ± 2.5
18:1(n-9)	27.3 ± 3.7	22.6 ± 2.3
18:1(n-7)	3.9 ± 0.2	2.9 ± 0.5
18:2(n-6)	14.1 ± 1.5	13 ± 1.2
20:3(n-9)	0.6 ± 0.06	0.6 ± 0.1
20:4(n-6)	7.4 ± 0.8	7.0 ± 1.6
22:6(n-3)	0.7 ± 0.1	0.7 ± 0.1
Classes		
Saturated	38.1 ± 6.0	43.7 ± 2.5
Monounsaturated	39.1 ± 5.5	31.1 ± 3.8
Polyunsaturated	22.8 ± 0.5	23.9 ± 0.6
(n-3)	0.9 ± 0.2	1.1 ± 0.2
(n-6)	22.5 ± 0.3	20.7 ± 1.4
Ratios		
sat/unsat	0.63 ± 0.16	0.81 ± 0.09

Results are means ± SE for four separate preparations of each membrane.

Table 4. Steady-state fluorescence polarization and steady-state fluorescence anisotropy of jejunal BBMVs prepared from SHR and WKY rats; r_s , fluorescence anisotropy

Parameter	WKY	SHR
P_{DPH}	0.342 ± 0.008	$0.262 \pm 0.006^*$
r_s	0.257 ± 0.007	$0.191 \pm 0.005^*$

Results are means \pm SE for at least ten different preparations.

* $p < 0.001$ compared to WKY rats.

P_{DPH} , fluorescence polarization.

Table, the major fatty acids present were palmitic (16:0), stearic (18:0), oleic (18:1, n-9) and linoleic (18:2, n-6). Arachidonic (20:4, n-6) and palmitoleic (16:1, n-7) acids ranged between 5 and 7% of the total fatty acids. High contents of fatty acids from the n-6 family were also found in both preparations. In contrast, n-3 fatty acids were noted in small amounts in BBMVs of both groups of animals. An increase, although not significant, was found in the ratio saturated/unsaturated fatty acid of SHR compared to WKY rats.

Membrane Fluidity

The steady-state fluorescence polarization (p) and fluorescence anisotropy (r_s) data for DPH-labeled jejunal brush-border membrane preparations from SHR and WKY rats are given in Table 4. There was a highly significant decrease in both parameters in brush-border membranes prepared from SHR, compared to those from WKY rats.

D-glucose and Na^+ uptake

Table 5 shows the initial rates of both Na^+ -dependent D-glucose uptake and pH-gradient dependent Na^+ uptake measured in SHR and WKY rats. The Na^+ -dependent D-glucose rate was increased 5.8-fold in SHR. However, the pH-gradient Na^+ uptake was only increased in SHR by 2.7-fold when compared to WKY rats.

Table 5. Na^+ -dependent D-glucose uptake and pH-gradient (pH 5.5 inside, 7.5 outside) Na^+ uptake in jejunal BBMVs prepared from SHR and WKY rats

	D-glucose uptake ($\text{pmol} \cdot \text{mg} \cdot \text{s}^{-1}$)	Na^+ uptake ($\text{pmol} \cdot \text{mg} \cdot \text{s}^{-1}$)
WKY	36 ± 7	5.5 ± 0.8
SHR	$209 \pm 32^{**}$	$15 \pm 3.4^*$

Results are means \pm SE for at least six different preparations.

* $p < 0.025$,

** $p < 0.001$ compared to WKY rats.

DISCUSSION

Our data indicate differences in the lipid composition of jejunal brush-border membranes prepared from SHR and WKY rats. Free cholesterol content of these membranes is increased in SHR compared to WKY rats. Since phospholipid levels were not altered by hypertension, a significant increment in the molar ratio of free cholesterol/phospholipid was observed in SHR. An increased cholesterol/phospholipid ratio has been previously reported in erythrocyte (9) and platelet (10) membranes of essential hypertensive patients.

To check whether the phospholipid composition changes in SHR, the phospholipid species were quantified. The predominant phospholipids in the control group, WKY rats, were PE and PS. This finding was not similar to that described by Bjorkman and Brigham (21) and Christon *et al.* (22), who indicated that PC together with PE were the predominant phospholipids in the whole small intestine from normotensive rats. These differences might be due to the portion of the intestine used, since our study was focussed only on the jejunum phospholipid composition. PE and PS were also the major phospholipids extracted from jejunum of SHR. However, PE levels were significantly decreased and PS levels increased in the SHR group. PC and SM levels did not significantly alter by hypertension, however, the PC/SM ratio was significantly increased in SHR.

The level of phosphatidylinositol (PI) found in the jejunal brush-border membrane prepared from SHR and WKY rats is less than 1%. It is accepted that inositol lipids are involved in signal transduction events and that a possible link between a global disturbance of cell membrane lipids and cell function, through membrane-based second messenger systems, may occur (23). In addition, disturbances of the phosphoinositide turnover have been reported in early stages of hypertension in SHR and man (24,25). However, we have not found differences in the level of PI in either group of animals.

Membrane fluidity (the inverse of microviscosity) is a property of the membrane that describes quantitatively the mobility and rate of rotational movement of molecules within the membrane. Membrane fluidity is decreased in erythrocyte (26,27), platelet (8) and vascular smooth muscle cell (7) membranes isolated from SHR. Devynck *et al.* (28) reported increments in microviscosity of the plasma membrane of SHR not only in erythrocytes, but also in hepatocytes, synaptosomes and cardiomyocytes, indicating that this membrane abnormality may affect the membrane structure and function of other tissues. Human essential hypertension is also characterized by increased membrane viscosity of erythrocyte and platelet membranes (7,29,30). However, studies on platelets have shown an increase in the membrane fluidity of hypertensive patients (31).

Our results show a significant decrease in both the steady-state fluorescence polarization (p) and fluorescence anisotropy (r_s) in SHR when compared to those from WKY rats, indicating that brush-border membranes from SHR showed a higher fluidity than those from WKY rats. Previous studies in model and biological membranes (32,33,34) have shown that differences in membrane fluidity may be secondary to alterations in the ratio cholesterol/phospholipid. An inverse relationship between brush-border membrane cholesterol/phospholipid

ratio and membrane fluidity has been demonstrated. However, in our case, this inverse relationship did not exist, since an increase in the cholesterol/phospholipid ratio in brush-border membranes prepared from SHR was accompanied by an increase in the membrane fluidity. The analysis of fatty acids in WKY rats revealed the presence of four major fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1 n-9) and linoleic (18:2 n-6), as previously reported for rats (35) and other animals (36). Hypertension did not produce large differences in the fatty acid composition of jejunal membranes, since it was similar for SHR. An increase, although not significant, in saturated-unsaturated ratio was also observed in SHR. Since the degree of fatty acyl chain unsaturation has been shown to be a determinant of membrane fluidity (37), these results do not explain the increased membrane fluidity (37), these results do not explain the increased membrane fluidity observed in SHR membranes. Consistent differences in the fatty acid composition of several tissues have been previously described between SHR and WKY rats, including renal medullary, renal cortical, brain and adrenal glands (11).

Concomitant with the changes in lipid composition and fluidity, we found an increase in both Na^+ -dependent D-glucose transport and Na^+ - H^+ antiport activity in the jejunal BBMV prepared from SHR when compared to WKY rats. These results are not in agreement with previous experiments in which a decrease in Na^+ -dependent D-glucose transport was noted in renal BBMV prepared from SHR (38). However, considerable evidence associates hypertension with the stimulation of the Na^+ - H^+ exchanger in lymphocytes (39), erythrocyte (40), platelet (41) and renal brush-border membranes (38) prepared from SHR. Similarly, essential hypertension in man is also characterized by an enhancement in Na^+ - H^+ exchanger (42).

Membrane lipid composition and fluidity can modulate the activity of ion transport pathways, including Na^+ - K^+ -ATPase, Na^+ - Ca^{2+} exchange, Ca^{2+} pump and Na^+ + H^+ exchanger (43, 44, 45). In addition, previous studies have demonstrated a direct correlation between fluidity and Na^+ -dependent D-glucose transport in rat small intestinal BBMV (46). Therefore, the differences in membrane lipid composition and fluidity noted between SHR and WKY rats could be responsible for alterations in membrane-bound proteins seen in hypertension. However, it is not clear whether altered membrane transport in SHR is due to extracellular factors or represents a basic plasma membrane abnormality as a metabolic consequence of the hypertensive status. Further studies are necessary to clarify these observations.

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