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LIPID DYNAMICS AND PROTEIN-LIPID INTERACTIONS IN RAT COLONIC EPITHELIAL CELL BASOLATERAL MEMBRANES

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Lipid dynamics and lipid-protein interactions were examined in basolateral membranes prepared from rat proximal and distal colonic epithelial cells. The results demonstrate that: (1) these membranes have a high lipid fluidity, as assessed by steady-state fluorescence polarization studies using seven fluorescent probes; (2) lipid compositional differences exist between these membranes but their fluidity is similar; (3) fluorescence polarization studies, using diphenylhexatriene (DPH), detect a thermotropic transition at 22–23°C in each membrane; (4) several membrane protein activities, including adenylate cyclase and sodium-potassium dependent adenosine triphosphatase ($(Na^+ + K^+)$-ATPase) appear to be functionally dependent on the physical state of the proximal basolateral membrane's lipid.

Introduction

The importance of lipid-protein interactions in influencing protein activities of biological membranes has received increased recognition in the past few years [1–3]. The characterization of lipid thermotropic transitions ** has provided a useful experimental tool for the elucidation of lipid-protein interactions in artificial and natural membranes [1,2,4,5]. In membranes with well defined lipid thermotropic transitions, i.e., *Escherichia coli* and *Mycoplasma*, it is well established that temperature-dependent changes in the physical state of the lipid influence protein-mediated membrane activities, particularly mechanisms involved in transmembranes transport [7–10]. Studies of isolated rat enterocyte [11–15], rat hepatocyte [6] and dog kidney plasma membranes [16] have provided clear evidence of lipid thermotropic transitions and their influence on mammalian membrane proteins. In rat enterocyte [13] and hepatocyte plasma membranes [6], differential scanning calorimetry has revealed broad lipid transitions of low enthalpy; fluorescence polarization studies detected the lower critical temperature of the transition; and Arrhenius plots of membrane protein activities revealed breakpoints corresponding to the lipid transition temperature. These latter protein functions have been operationally classified as 'intrinsic activities' since they appear to be dependent on the physical state of membrane lipids [12–15].

The mammalian colon performs important

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** The term 'lipid thermotropic transition' is used in a general sense to denote a thermally induced change in the physical state of the lipid. This change might involve order-disorder transitions of the liquid crystalline to gel type, lipid clusters, lateral phase separations, or other mechanisms [4–6].

physiological functions, many of which are protein mediated, and yet to date, little is known about lipid-protein interactions or lipid dynamics in colonic epithelial cell plasma membranes. This present report focuses on the lipid-protein interactions and lipid dynamics of basolateral membranes prepared from rat proximal and distal colonic segments. The results described below demonstrate: (1) basolateral membranes from rat colon possess a relatively high lipid fluidity *, as assessed by fluorescence polarization studies using seven fluorescent probes; (2) lipid compositional differences exist between proximal and distal colonic basolateral membranes but their fluidity is not significantly different; (3) a lipid thermotropic transition is present in each membrane, as assessed by fluorescence polarization using the fluorophore 1,6-diphenyl-1,3,5-hexatriene (DPH); (4) Arrhenius plots of several membrane protein activities, including sodium-potassium dependent adenosine triphosphatase ($(Na^+ + K^+)$-ATPase) and adenylate cyclase, demonstrate breakpoints corresponding to the transition temperature and therefore can be operationally classified as 'intrinsic membrane activities'.

Methods and Materials

Isolation of epithelial cells from proximal and distal colonic segments

Male albino rats of the Sherman strain, weighing 250–300 g, were used for all studies. The animals were fasted 18 h with water ad libitum before being killed. The animals were killed rapidly by cervical dislocation and their colons excised. The cecum was discarded and the remaining large intestine equally divided into two parts: a 'proximal' and 'distal' portion. Cells were obtained using a modification of a method previously described [24] as follows: each segment was rinsed thoroughly with a solution containing 0.015 M NaCl and 0.001 M dithiothreitol, and tied at one end. The sac was filled, tied at the other end and shaken at 37°C in a 250 ml siliconized glass Erlenmeyer flask containing 50 ml of phosphate-buffered saline, pH 7.2, at 75 oscillations per min, sequentially, with the following solutions: 10% fetal calf serum in 1.6% Joklik's modified minimal essential medium (Solution A) (GIBCO: Grand Island, NY) × 10 min (twice); citrate buffer (Solution B) (0.027 M sodium citrate; 0.0015 M KCl; 0.096 M NaCl; 0.008 M KH_2PH_4; 0.0056 M Na_2HPO_4, pH 7.3) × 15 min; 0.1 M phosphate-buffered saline, pH 7.2, containing 0.0015 M EDTA and 0.0005 M dithiothreitol (Solution C) × 15 min (twice).

Each solution was collected, filtered through a single layer of Nitex (Tetko, Inc., Elmsford, NY) with a pore diameter of 100 μm, then each was brought up to 40 ml with phosphate-buffered saline (pH 7.2) (containing 0.001 M Ca^{2+} and 0.001 M Mg^{2+}) and centrifuged at 500 g × 10 min. The cell pellets were washed twice in the same buffer and kept on ice until used (see below).

Basolateral membrane preparations

Basolateral membranes were prepared as follows: the cells from all five incubations from each segment of rat colon were combined, pelleted, washed and suspended in 50 ml of sucrose-histidine-imidazole-EDTA Buffer (Solution D) (0.25 M sucrose; 0.005 M histidine; 0.005 M imidazole; 0.005 M EDTA, pH 7.0). Each suspension of cells (proximal and distal) was then homogenized × 20 strokes, using a tight fitting Dounce homogenizer (pestle A), filtered through a single layer of Nitex (Tetko, Inc., Elmsford, NY) with a pore diameter of 100 μm to remove debris and unbroken cells, and washed with an additional 10–20 ml of Solution D. The homogenate was centrifuged at 300 g × 10 min at 4°C in a Sorvall Centrifuge (Dupont Instruments, Model RC-5B). The supernatant was collected and kept on ice while the pellet was

* The term 'lipid fluidity' as applied to model bilayers and natural membranes is used to express the relative motional freedom of the lipid molecules or substituents thereof. It bears emphasis, however, that this term is broad and includes different types of motion, e.g., rotational or lateral diffusion of a molecule in an array, movements of substituent groups of a molecule, and flow of molecules under a pressure gradient in accord with a fluidity which is 1/viscosity of the molecular array [17–19]. In this report 'lipid fluidity' was assessed by the steady-state fluorescence polarization of lipid soluble probes. The anisotropy parameters so obtained are probe dependent and reflect the overall motional freedom of these molecules without distinguishing the specific mechanism affecting its motion such as viscous drag of the environment, anisotropic rotations and hindered motions due to structural factors [14,20–23].

brought up in 10 ml of Solution D, dounced × 10 strokes and spun again at 300 $g \times 10$ min. The supernatant was again collected. The pellet was brought up in 10 ml of the same buffer, dounced × 5 strokes and centrifuged at 360 $g \times 10$ min. The supernatant was combined with the first two supernatants and centrifuged at 1085 $g \times 15$ min. The pellet was discarded and the supernatant then centrifuged at 22000 $g \times 15$ min. The 'fluffy white portion of the pellet' was collected, brought up in 5 ml of 50% sucrose/Solution D (w/w) and dounced × 5 strokes. 4 ml of this material were placed at the bottom of a gradient made up with 3.0 ml of 40% sucrose/Solution D, 3.0 ml of 30% sucrose/Solution D and filling the remainder of the tube with 20% sucrose/Solution D (w/w). The discontinuous gradient was centrifuged at 140000 $g \times 120$ min in a Beckman SW 36 rotor using a Beckman L5-50 ultracentrifuge (Palo Alto, CA). The 30/40 fraction of each segment was collected, diluted with distilled H_2O and pelleted by ultracentrifugation, using a Beckman 40 rotor, at 105000 $g \times 60$ min. Each pellet was then brought up in 1 ml of Solution D, pH 7.4 and analyzed immediately or quick frozen, with acetone and dry ice, and stored at −70°C.

Enzyme assays

Assay conditions were chosen for each enzyme to assure linear kinetics with respect to time and protein. Adenylate cyclase, $(Na^+ + K^+)$-ATPase, magnesium-dependent adenosine triphosphatase (Mg^{2+}-ATPase), NADPH-cytochrome *c* reductase, succinate dehydrogenase and alkaline phosphatase (*p*-nitrophenylphosphatase) were assayed as previously described [12–14]. The latter enzyme was also measured using the fluorogenic substrate 4-methylumbelliferyl phosphate [25]. Acid phosphatase, ouabain-sensitive and -insensitive potassium-dependent phosphatases were measured as previously described [25]. Exogeneous galactosyltransferase was measured according to the method of Podolsky and Weiser [26], using ovalbumin as exogeneous acceptor. Arrhenius plots were determined for adenylate cyclase, $(Na^+ + K^+)$-ATPase, Mg^{2+}-ATPase and ouabain-sensitive K^+-dependent phosphatase as previously described [11–15]. V_{max} conditions were always used.

Lipid extracts and liposomes

Total lipids were extracted from the membranes by the method of Folch et al. [27]. Sonicated dispersions of extracted lipid (liposomes) were prepared as previously described [12].

Fluorescence polarization studies

Seven fluorophores were used: 1,6-diphenyl-1,3,5-hexatriene (DPH), all-*trans*-retinol (R), DL-2-(9-anthroyl)stearic acid (2-AS), DL-7-(9-anthroyl)stearic acid (7-AS), DL-9-(9-anthroyl)stearic acid (9-AS), DL-12-(9-anthroyl)stearic acid (12-AS), and DL-16-(9-anthroyl)stearic acid (16-AS). All compounds were obtained from Aldrich Chemical Co., Sigma Chemical Co., or Molecular Probes Inc. The methods used to load the membranes and the quantification of the polarization of fluorescence in an SLM polarization spectrofluorometer have been described [11–15].

The polarization of fluorescence was expressed as the fluorescence anisotropy parameter. $((r_0/r) - 1)^{-1}$, which within the limitations discussed previously [14] is proportional to the apparent rotational relaxation time of the probe. The temperature dependence of $((r_0/r) - 1)^{-1}$ was determined over the range of 0 − 40°C and 0 − 50°C, respectively, for membranes and liposomes using DPH. The logarithm of the anisotropy parameter was plotted against 1/K, as previously described [11,13,28] to detect thermotropic transitions. No changes in the excited state lifetime, as assessed by total fluorescence intensity, were demonstrated using each probe in either basolateral membrane or liposome preparations.

Composition studies

Protein was estimated by the method of Lowry et al. [29]. Total lipids were extracted from membrane preparations by the method of Folch et al. [27]. Cholesterol and phospholipid were measured by the methods of Zlatkis et al. [30] and Ames and Dubin [31], respectively. The lipid composition of the extracts was further examined by thin-layer chromatography according to the procedure of Katz et al. [32]. Fatty acids of the total lipid extracts were derivatized as described by Gartner and Vahouny [33]. Fatty acid methyl esters were determined on a Joel JGC-20 K gas chromato-

graph equipped with a flame ionization detector and interfaced with a Hewlett-Packard 3390A intergrator, using authentic fatty acid methyl esters to identify retention times [33].

Materials and general methods

All radioactive materials were purchased from New England Nuclear Company. NCS tissue solubilizer was obtained from Amersham Company. Fatty acids, methyl esters, GLC columns and lipid standards were purchased from Applied Science Corp. and or Supelco. All other materials were obtained from either Fisher Chemical Co. or Sigma Chemical Co. DNA content was measured using the method of Burton [34] as modified by Giles and Myers [35].

Statistical methods

All results are expressed as mean values ± S.E. Paired or unpaired *t*-tests were used for all statistical analysis. A P value < 0.05 was considered significant.

Results

Preparation of basolateral membrane fractions

Histologic examination of the cells harvested from each segment of rat colon demonstrated that greater than 90% were epithelial cells and only 5–10% were mucus-secreting cells (goblet cells). 90% of these cells excluded Trypan blue dye. These cells were used as starting material from which basolateral membranes were isolated.

The purity and comparability of basolateral membranes from each colonic segment were assassed by estimation of the specific activities of the marker enzymes $(Na^+ + K^+)$-ATPase and ouabain-sensitive K^+-dependent phosphatase. Membrane preparations were purified 12- to 15-fold as compared to starting homogenates.

The basolateral membrane preparations were found not to be significantly contaminated by microsomal, mitochondrial, lysosomal, Golgi, nuclear or lumenal plasma membranes using the enzyme markers NADPH-cytochrome *c* reductase, succinate dehydrogenase, acid phosphatase, galactosyltransferase, DNA and cysteine inhibitable alkaline phosphatase and ouabain-insensitive K^+-dependent phosphatase, respectively (data not shown).

Fluorescence polarization studies

The effects of temperature on the anisotropy parameter, $((r_0/r) - 1)^{-1}$, of DPH, in basolateral membranes and liposomes prepared from the membrane lipids of proximal and distal colonic epithelial cells are illustrated by representative Arrhenius plots in Fig. 1. The plots appear rea-

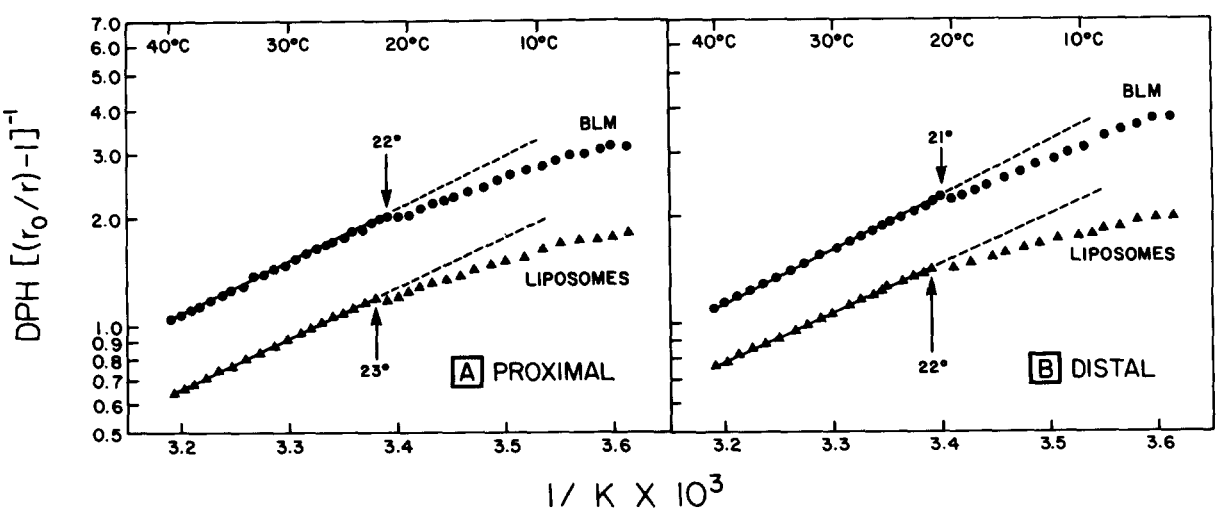

Fig. 1. Arrhenius plots of the anisotropy parameter of diphenylhexatriene in a sample of isolated basolateral membranes (BLM, upper curve) and in liposomes prepared from a lipid extract of these membranes (lower curve) prepared from (A) proximal rat colonic epithelial cells; (B) distal rat colonic epithelial cells.

TABLE I

THERMOTROPIC TRANSITION TEMPERATURES AND DIPHENYLHEXATRIENE (DPH) ANISOTROPY PARAMETERS IN MEMBRANES AND LIPOSOMES

Values presented are means ± S.E. *N*, number of preparations.

Preparations	*N*	Transition temp. (°C)	DPH$((r_0/r)-1)^{-1}$	
			25°C	37°C
Proximal membranes	11	22 ± 1.8	1.80 ± 0.26	1.18 ± 0.10
Proximal liposomes	3	23 ± 2.3	1.11 ± 0.15	0.72 ± 0.09
Distal membranes	5	21 ± 2.1	1.92 ± 0.31	1.23 ± 0.12
Distal liposomes	3	22 ± 1.6	1.28 ± 0.10	0.84 ± 0.11

sonably linear from 40°C to approximately 21–23°C, but a distinct change in slope then occurs with further cooling and the plots become somewhat curvilinear. The results summarized in Table I indicate that the transition temperatures are similar for both membranes and their liposomes. The anisotropy parameter values for each proximal membrane and liposome are not significantly different from their distal counterparts, using DPH. The anisotropy parameters of DPH, at 25 and 37°C are, however, approximately twice as great for the basolateral membrane preparations compared to their respective liposomes, indicating that the fluidity of the lipids is considerably greater in the liposomes.

To further examine whether differences in lipid fluidity might exist between membranes and liposomes prepared from each colonic segment and to overcome some limitations in the technique as previously discussed [14], additional studies were conducted using six other fluorescent probes which differ in structure and shape and localize in various domains of the bilayer. In each case (Table II), the anisotropy parameter for the membranes was higher than their liposomes, further demonstrating that membrane proteins decreased the lipid fluidity of these membranes. Comparison of the anisotropy parameters of the proximal membranes and liposomes with their respective distal counterparts again revealed no significant differences with any of the six probes (Table II).

As can be seen in Table II, the anisotropy values for 2-AS and 7-AS, in membranes and liposomes, exceeded the values obtained with the other anthroyl series of probes. These probes appear to locate in a graded series of depths from the surface to the center of the lipid bilayer and have been used to measure fluidity gradients in liposomes and natural membranes [36,37] and in multilamellar vesicles of dipalmitoylphosphatidylcholine [38]. In agreement with previous studies it appears that 2-AS and 7-AS experience less freedom of motion than 9-AS, 12-AS or 16-AS, presumably because the former probes are localized in the bilayer closer to the aqueous interface [36–38]. 7-AS experiences the least freedom of motion apparently because it is in the region of the double bonds of unsaturated phospholipids [36,37].

Temperature dependence of enzyme activities

A number of proximal colonic basolateral membrane enzyme activities were examined to detect breakpoints in their Arrhenius plots and to explore the relationship of such breakpoints to the lipid thermotropic transition temperature. Four membrane preparations were examined for Mg^{2+}-ATPase, adenylate cyclase (basal and stimulated with NaF), $(Na^+ + K^+)$-ATPase and ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity. The latter two activities are mediated by the same enzyme complex [39], which comprises the ouabain-sensitive cation pump responsible for active extrusion of intracellular Na^+ in exchange for extracellular K^+. Each preparation and each activity tested demonstrated a discontinuity in Arrhenius plot, as illustrated by plots of the mean values seen in Fig. 2. The apparent breakpoint temperatures and energies of activation, *E*, for these experiments are summarized in Table III. The break points observed for basal and NaF

TABLE II

FLUORESCENCE POLARIZATION STUDIES

P values for the differences between intact basolateral preparations and their respective liposome preparations are indicated. *N*, number of preparations.

Probe	Proximal (P) and distal (D)	*N*	Anisotropy parameter at 25°C [a]	*P*	Mean fluorescence anisotropy, *r*, at 25°C
Retinol					
	(P) Membrane	4	2.22 ± 0.13	< 0.05	0.253
	(P) Liposomes	3	1.84 ± 0.03		0.238
	(D) Membrane	4	2.25 ± 0.19	< 0.05	0.254
	(D) Liposomes	3	1.91 ± 0.09		0.241
2-AS					
	(P) Membrane	5	0.45 ± 0.04	< 0.01	0.089
	(P) Liposomes	3	0.30 ± 0.03		0.066
	(D) Membrane	5	0.41 ± 0.04	< 0.01	0.083
	(D) Liposomes	3	0.30 ± 0.05		0.066
7-AS					
	(P) Membrane	4	0.49 ± 0.04	< 0.01	0.094
	(P) Liposomes	3	0.34 ± 0.02		0.072
	(D) Membrane	4	0.45 ± 0.04	< 0.01	0.088
	(D) Liposomes	3	0.33 ± 0.02		0.071
9-AS					
	(P) Membrane	5	0.39 ± 0.02	< 0.01	0.080
	(P) Liposomes	3	0.28 ± 0.01		0.062
	(D) Membrane	5	0.38 ± 0.02	< 0.01	0.079
	(D) Liposomes	3	0.28 ± 0.02		0.062
12-AS					
	(P) Membrane	3	0.32 ± 0.02	< 0.05	0.069
	(P) Liposomes	3	0.27 ± 0.01		0.060
	(D) Membrane	3	0.31 ± 0.02	< 0.05	0.068
	(D) Liposomes	3	0.25 ± 0.02		0.057
16-AS					
	(P) Membrane	3	0.28 ± 0.04	< 0.05	0.062
	(P) Liposomes	3	0.23 ± 0.01		0.053
	(D) Membrane	3	0.28 ± 0.01	< 0.05	0.062
	(D) Liposomes	3	0.23 ± 0.02		0.053

[a] Values presented are means ± S.E.

stimulated adenylate cyclase and Mg^{2+}-ATPase are similar, i.e., approx. 25–28°C. The breakpoints for $(Na^+ + K^+)$-ATPase and ouabain-sensitive K^+-dependent phosphatase were found to be approx. 19–20°C. The latter breakpoints are some 2–3 K below the phase transition temperature determined by fluorescence polarization in these membranes (22°C) and 6–8 K below the corresponding breakpoints observed for Mg^{2+}-ATPase and adenylate cyclase.

Membrane composition

While no differences in lipid fluidity appeared to exist between basolateral membranes prepared from rat proximal and distal colon, both membranes possessed a relatively high lipid fluidity. In this regard, prior studies in model bilayers and natural membranes have correlated a high lipid fluidity with low molar ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine [40–43]. A low ratio of protein/lipid (w/w) [11,

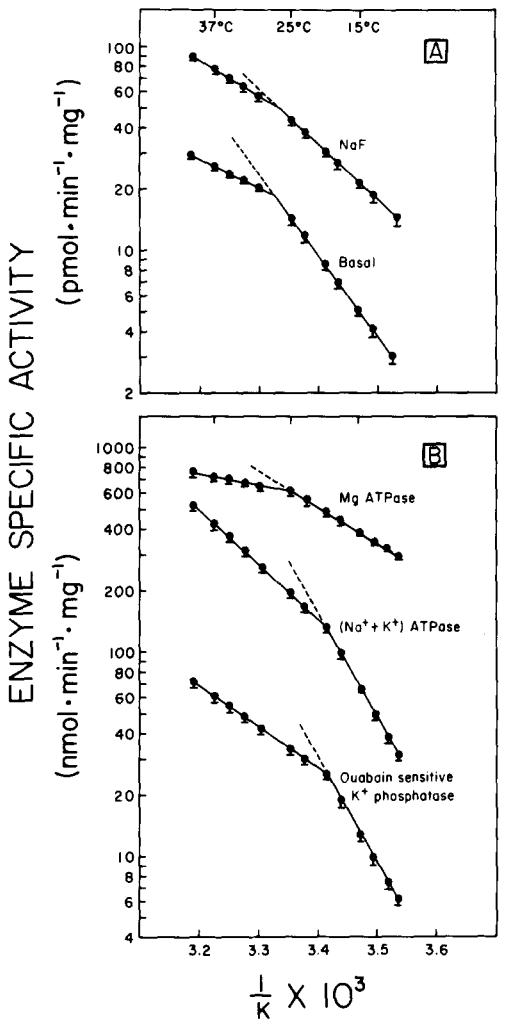

Fig. 2. Arrhenius plots of rat proximal basolateral membrane (A) basal and NaF-stimulated adenylate cyclase; (B) Mg^{2+}-ATPase (upper curve), $(Na^+ + K^+)$-ATPase (middle curve) and ouabain-sensitive K^+-dependent phosphatase (lower curve). Table III lists the number of preparations, the apparent energies of activation, and the temperature of the breakpoints.

TABLE IV

COMPOSITION OF LIPID EXTRACTS OF RAT BASOLATERAL MEMBRANES

Values presented are means ± S.E. for lipid extracts for four preparations each of proximal and distal colonic basolateral membranes.

Component	% (w/w) total lipid of basolateral membranes	
	Proximal	Distal
Cholesterol	11.9 ± 1.1	12.6 ± 0.5
Cholesterol esters	1.6 ± 0.5	1.2 ± 0.6
Triacylglycerols	0.5 ± 0.3	0.3 ± 0.1
Fatty acids	12.4 ± 2.1	11.2 ± 2.3
Phosphatidylcholine	36.1 ± 1.1	36.9 ± 1.4
Lysophosphatidylcholine	0.2 ± 0.1	0.1 ± 0.1
Sphingomyelin	13.1 ± 2.6	19.1 ± 1.0 [a]
Phosphatidylethanolamine	25.9 ± 1.9 [b]	19.4 ± 2.3

[a] $P < 0.05$ compared to proximal value.
[b] $P < 0.05$ compared to distal value.

TABLE III

TEMPERATURE DEPENDENCE OF PROXIMAL COLONIC BASOLATERAL MEMBRANE ENZYME ACTIVITIES

Values presented are means ± S.E. *N*, number of preparations.

Enzyme activity	*N*	T_b (°C)	Apparent energy of activation, ΔE (kcal/mol)	
			below T_b	above T_b
Adenylate cyclase				
Basal	4	27.1 ± 1.6	19.8 ± 1.9	12.8 ± 1.3
NaF (15 mM)	4	28.0 ± 1.5	14.5 ± 1.8	9.3 ± 1.6
Mg^{2+}-ATPase	4	25.2 ± 1.4	9.3 ± 0.7	2.3 ± 0.8
$(Na^+ + K^+)$-ATPase	4	18.8 ± 1.6	26.8 ± 3.1	14.0 ± 2.0
Ouabain-sensitive K^+-dependent phosphatase	4	20.1 ± 1.5	26.5 ± 3.6	11.4 ± 2.1

TABLE V

COMPOSITION OF TOTAL FATTY ACIDS OF RAT COLONIC BASOLATERAL MEMBRANES

Fatty acid	% (mass/mass)	
	Proximal	Distal
12:0	1.11 ± 0.32	1.34 ± 0.18
12:1	0.31 ± 0.09	0.58 ± 0.10 [a]
14:0	1.58 ± 0.20	2.29 ± 0.25
14:1	0.98 ± 0.18	1.20 ± 0.16
16:0	28.24 ± 0.85	28.59 ± 0.98
16:1	2.12 ± 0.12	4.98 ± 0.50 [a]
18:0	12.21 ± 0.38	13.49 ± 0.98
18:1	24.74 ± 0.89	26.55 ± 0.69 [a]
18:2	12.72 ± 0.91 [b]	9.20 ± 0.72
20:0	0.32 ± 0.21	0.38 ± 0.19
20:1	0.33 ± 0.33	0.51 ± 0.25
20:3	1.87 ± 0.23	2.63 ± 0.62
20:4	9.32 ± 0.64 [b]	7.76 ± 0.74

[a] $P < 0.05$ or greater compared to proximal colon.
[b] $P < 0.05$ or greater compared to distal colon.

13] as well as less saturated or shorter acyl chains in phospholipid [43,44] also appears to be associated with higher lipid fluidity. It was, therefore, of interest to examine these parameters in both membranes. In four preparations of basolateral membranes of rat proximal and distal colon, the ratios of protein/lipid (w/w), respectively, were 1.60 ± 0.11 and 1.83 ± 0.20 ($P > 0.05$). The lipid extracts of each membrane were examined by thin-layer chromatography and the results are summarized in Table IV. The cholesterol/phospholipid molar ratios of both membranes were 0.4 ± 0.1. The molar ratios of sphingomyelin/phosphatidylcholine in proximal and distal colonic membranes, respectively, were 0.39 ± 0.02 and 0.52 ± 0.03 ($P < 0.05$). The fatty acid composition of the total lipid extract for each membrane are presented in Table V. The proximal colonic membrane possessed fatty acids with longer chains although no difference in the ratio (mass/mass) of saturated to unsaturated fatty acids was present.

Discussion

The present results demonstrate that a lipid thermotropic transition can be detected in basolateral membranes prepared from rat proximal and distal colonic epithelial cells, by steady-state fluorescence polarization using the fluorophore DPH, at 22–23°C. While these temperatures are well below 37°C, a physiological role for this change in the physical state of the membrane lipid is not precluded. Previous studies in our laboratory have demonstrated that this method detected only the lower critical temperature of broad transitions observed by differential scanning calorimetry in rat enterocyte [13] and hepatic plasma membranes [6]. The transition temperatures detected in the rat colonic basolateral membranes in the present studies therefore probably represent the lower critical temperature of the lipid transition *. The characteristics of the particular method employed may influence the likehood of observing the lower versus the upper critical temperature of the membrane lipid transition [6]. In our studies DPH was utilized. This probe partitions equally between gel and liquid crystalline phases of model bilayer membranes [45]. Differential polarized phase fluorimetry studies, in model bilayers using this probe, have shown that the degree of hinderance of its rotation, more than its rate of rotation is altered by the physical state of the lipid [18,19]. As previously discussed [6], this suggests that the hinderance of the DPH rotations is sensitive to changes in the lipid organization which occurs at the lower critical temperature.

The present studies also demonstrate that the membrane lipid may influence certain protein-dependent activities, namely, Mg^{2+}-ATPase, adenylate cyclase (basal and NaF stimulated), ($Na^+ + K^+$)-ATPase and ouabain-sensitive K^+-dependent phosphatase. Arrhenius plots of these activities demonstrated breakpoints in the vicinity of the lipid thermotropic transition temperatures. While temperature-sensitive changes in protein conformation may be independent of the membrane lipids [46], all studies were performed under maximum velocity conditions and constant pH to preclude artifactual breaks in slope. Furthermore,

* Evidence for detection of both upper and lower critical temperatures of a lipid transition by Arrhenius plots of the anisotropy parameter of DPH have been described in dog kidney membrane liposomes but not in the membranes themselves [16]. However, we are thus far unable to detect the upper critical temperature of the transition in either hepatic, enterocyte or colonic membranes or their liposomes by comparable experiments.

previous studies [12] have demonstrated removal of the breakpoint seen in Arrhenus plots of the rat enterocyte microvillus membrane's *p*-nitrophenylphosphatase by delipidation and restoration of the original breakpoint temperature by relipidation. This suggests that these four activities are 'intrinsic membrane activities' which are mediated by proteins which are influenced by the physical state of their membrane lipid. As a group, with the possible exception of Mg^{2+}-ATPase, these intrinsic membrane activities may share a common function: transmembrane transport of mass or information [12,15].

These enzymes can be further subdivided into two groups. Mg^{2+}-ATPase and adenylate cyclase (basal and stimulated) demonstrate breakpoints in their plots at temperatures (15–28°C) slightly above the lipid transition detected by studies using DPH, whereas, $(Na^+ + K^+)$-ATPase and ouabain-sensitive K^+-dependent phosphatase, show breaks in their slopes (19–20°C), some 2–3 K below the transition temperature. Why certain colonic membrane activities should exhibit different breakpoints above and below the lipid thermotropic transition temperature is unclear, although similar findings have been reported for rat enterocyte basolateral [14], rat hepatocyte [6], *Mycoplasma* [1,47] and *Escherichia coli* [2,48] membranes. Presumably, the lipid microenvironment around a particular intrinsic enzyme and the nature of the specific protein-lipid interactions may influence the breakpoint temperature [14,49–51]. Specifically, with regard to $(Na^+ + K^+)$-ATPase, similar results have previously been shown for both rat enterocyte basolateral [14] and rabbit kidney microsomal membranes [52]. This suggests that this enzyme may function in a lipid environment which is more fluid than the bulk lipid and requires a lower temperature for the transition. Grisham and Barnett [53] and Kimelberg and Papahadjopoulous [52] have, in fact, demonstrated that the lipid associated with purified lamb kidney $(Na^+ + K^+)$-ATPase was more fluid than that present in crude microsomal starting mterial. That $(Na^+ + K^+)$-ATPase may not universally require a more fluid environment, however, was shown by the studies of LeGrimellec et al. [16]. These investigators demonstrated a breakpoint in Arrhenius plot for this enzyme at 21°C in dog kidney basolateral membranes which possessed a lower critical transition temperature of 17°C.

Prior fluorescence polarization studies from our laboratory [14] have revealed that the motional freedom of lipid molecules was considerably greater in basolateral than in microvillus membranes prepared from rat enterocytes. This has subsequently been confirmed using electron spin resonance techniques [54]. Basolateral membranes of dog kidney epithelial cells also appear to possess a higher lipid fluidity than their lumenal counterparts [16], suggesting that this gradient of fluidity may be a general phenomenon. Furthermore, the lipid fluidity of both basolateral [55] and microvillus [11,56] membranes appears to decrease along the length of the small intestine. The fluidity gradient, however, between these antipodal membranes is still maintained. Compositional differences between these membranes appear to explain these differences in lipid dynamics [14].

The present data demonstrate that both proximal and distal colonic basolateral membranes possess a high lipid fluidity, in fact, similar to that previously shown for rat enterocyte basolateral membranes [14]. This high fluidity appears to result from low ratios of cholesterol/phospholipid (mol/mol), sphingomyelin/phosphatidylcholine (mol/mol) and protein/lipid (w/w) present in these membranes [11,13,40–43]. No significant differences in lipid fluidity appear to exist between membranes prepared from proximal and distal colonic epithelial cells. Despite this latter finding, certain compositional differences were shown to exist between these two membranes. Fatty acids with longer chains were present in the proximal membrane, whereas, the sphingomyelin/phosphatidylcholine molar ratio was significantly greater in the distal membrane. Theoretically, each of these differences should produce a lower lipid fluidity in their respective membranes. Since no difference in fluidity could be detected, however, this data suggest that these differences either did not influence the lipid fluidity or more likely that they served to offset each other, thereby, maintaining a relatively constant fluidity in basolateral membranes along the length of the colon. The high lipid fluidity in both membranes as well as differences in lipid composition between them may have functional significance. Further studies of

these membranes should clarify questions concerning possible functions and mechanisms for maintaining these differences in lipid composition.

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