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The lipid fluidity of rat colonic brush-border membrane vesicles modulates Na^+-H^+ exchange and osmotic water permeability

Thomas A. Brasitus [a,b,*], Pradeep K. Dudeja [a], Howard J. Worman [b,**] and Emily S. Foster [a,b]

[a] *Department of Medicine, Michael Reese Hospital and Medical Center, Chicago, IL 60616 and* [b] *Pritzker School of Medicine, University of Chicago, Chicago, IL 60637 (U.S.A.)*

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Brush-border membrane vesicles were prepared from rat colonic epithelial cells. Steady-state fluorescence polarization techniques, using the fluorophores 1,6-diphenyl-1,3,5-hexatriene and DL-12-(9-anthroyl)stearic acid (12-AS), revealed that benzyl alcohol (25–75 mM) but not methyl alcohol (50–125 mM) significantly increased the fluidity of these vesicles. Benzyl alcohol (50 and 75 mM) but not methyl alcohol also increased amiloride-sensitive sodium-stimulated proton efflux from these vesicles at all concentrations of sodium tested (2.5–50.0 mM), as assessed by changes in the fluorescence of acridine orange. Benzyl alcohol, at 50 and 75 mM concentrations, increased the maximal velocity (V_{max}) of this exchange process by approximately 58 and 75%, respectively. Neither concentration, however, altered the K_m for sodium. Osmotic water flow, measured as rate constants of osmotic shrinkage of these vesicles using a stopped-flow nephelometric technique, was also increased by 75 mM benzyl alcohol but not by a similar concentration of methyl alcohol. The present data, therefore, demonstrate that the fluidity of rat colonic brush-border membranes can influence Na^+-H^+ exchange and osmotic water flow across these vesicles.

1. Introduction

There is considerable evidence that many important functions of biological membranes are influenced by the physical state of the membrane lipid [1–3]. In recent years, our laboratory has described studies of the lipid dynamics and lipid-protein interactions in plasma membranes prepared from rat small intestinal [4–10] and colonic [11–13] epithelial cells. A number of activities, including certain transmembrane transport processes, have been shown to be influenced by the lipid fluidity *** of these membranes [4,7].

* To whom correspondence should be addressed at: Division of Gastroenterology, Department of Medicine, Michael Reese Hospital and Medical Center, Chicago, IL 60616, U.S.A.

** Present address: Department of Medicine, Cornell University Medical College, New York, NY 10021, U.S.A.

*** The term 'lipid fluidity' as applied to anisotropic bilayer membranes is used in this paper to denote the relative motional freedom of the lipid molecules or substituents thereof. A more detailed description has been published [27]. Briefly, as evaluated by steady-state fluorescence polarization of lipid fluorophores, 'fluidity' is assessed by the parameters of the modified Perrin equation described under Materials and Methods. An increase in fluidity corresponds to a decrease in either the correlation time, T_c, or the hindered anisotropy, r_∞, of the fluorophore, thereby, combining the concepts of the dynamic and static (lipid order) components of fluidity.

The mammalian colon performs several important physiological functions including maintenance of normal electrolyte and water balance [14]. In vivo, the large intestine absorbs sodium, chloride and water and secretes potassium and bicarbonate [14]. Recently, our laboratory has identified and partially characterized Na^+-H^+ exchange process in rat colonic brush-border membrane vesicles, using the pH-sensitive fluorescent dye acridine orange [15]. This process appears to play an important role in electroneutral sodium absorption, the predominant sodium absorptive process in the large intestine of the rat [14]. To date, the possible influence of membrane lipid fluidity on this exchange process has not been examined.

Similarly, prior studies in model membranes have suggested that their lipid composition and fluidity may influence the water permeability of these membranes [16–18]. Recent experiments in our laboratory of osmotic water flow across rat small intestinal brush-border membrane vesicles, as assessed by a stopped-flow nephelometric technique, also suggest that the lipid fluidity of these membranes affects their water permeability properties [19].

It was, therefore, of interest to examine whether Na^+-H^+ exchange as well as osmotic water flow could be influenced by alterations in the lipid fluidity of rat colonic brush-border membrane vesicles induced by benzyl alcohol. This local anesthetic readily partitions into aqueous solutions and has previously been shown to increase the fluidity of model bilayer [20] and biological membranes [5,21]. The studies demonstrate that benzyl alcohol-induced changes in the lipid fluidity of rat colonic brush-border membrane vesicles correlated with alterations in Na^+-H^+ exchange and osmotic water flow.

Materials and Methods

Preparation of colonic brush-border membrane vesicles

Male albino rate of the Sherman strain weighing 250–300 g were fasted 18 h with water ad-libitum before being killed. The colons were excised, the cecum discarded and epithelial cells, relatively devoid of goblet cells, were obtained using a technique which combined chelation of divalent cations with mild mechanical dissociation as described [11].

Brush-border membrane vesicles from epithelial cells were then prepared as reported by Brasitus and Keresztes [13]. The purity of the membrane suspensions and the degree of contamination with intracellular organelles were assessed by marker enzymes. The specific activity ratios [(purified brush-border membrane)/(crude homogenate)] for the brush-border enzyme markers, total alkaline phosphatase (*p*-nitrophenylphosphatase) and cysteine-sensitive alkaline phosphatase, were approximately 15–20 in all membrane preparations. The corresponding values for succinate dehydrogenase, NADPH:cytochrome-*c* reductase and sodium-potassium-dependent adenosine triphosphatase, marker enzymes for mitochondrial, microsomal and basolateral membranes, respectively, ranged from 0.50 to 1.50 in all membrane preparations. Brush-border membrane vesicles were suspended in the appropriate buffer (see below) and used immediately.

Fluorescence polarization studies

Two fluorophores were used: 1,6-diphenyl-1,3,5-hexatriene (DPH) and DL-12-(9-anthroyl)-stearic acid (12-AS). These compounds were obtained from Aldrich Chemical Co. or Molecular Probes Inc. Steady-state fluorescence polarization studies were performed with a Perkin-Elmer 650–40 spectrofluorometer adapted for fluorescence polarization. The methods used to load the membranes and the quantification of the polarization of fluorescence have been described [4–9,13]. The content of each fluorophore in the membranes was estimated fluorometrically as described by Cogan and Schachter [22]. Final molar ratios of probe/lipid ranged from 0.001 to 0.002, and the anisotropy variations noted in these studies could not be ascribed to differences in probe concentrations in the membranes. Corrections for light scattering (preparations minus probe) and for fluorescence in the ambient medium (quantified by pelleting the preparations after each estimation) were made routinely; the combined corrections were less than 3% of the total fluorescence intensity observed for diphenylhexatriene-loaded preparations and less than 5% of that observed for

anthroyloxystearate-loaded suspension. The results were obtained according to the modified Perrin relationship [23–25]

$$r = r_\infty + (r_0 - r_\infty)[T_c/(T_c + T_f)]$$

where r is the fluorescence anisotropy; r_0, the maximal limiting anisotropy; taken as 0.365 for diphenylhexatriene [26] and 0.285 for 12-AS [27]; r_∞, the limiting hindered anisotropy; T_c, the correlation time; and T_f, the mean lifetime of the excited state. Values of r_∞ for diphenylhexatriene were calculated from r values as previously described by Van Blitterswijk et al. [25]. The static component of membrane fluidity was assessed by an order parameter, S, where $S = (r_\infty/r_0)^{1/2}$ as described previously [23–25]. No change in the excited state-lifetimes, as assessed by total fluorescence intensity, was demonstrated for the two probes in the presence and absence of benzyl or methyl alcohol in each preparation examined [9,10,12,13].

Na^+-H^+ exchange studies

Fluorescent dye experiments. Utilizing the quenching of acridine orange fluorescence to monitor changes in the transmembrane pH gradient as recently described by our laboratory and others [15,28–30], the effect of external Na^+ on proton efflux was evaluated. The fluorescence of acridine orange was measured at 26°C with a Perkin-Elmer 650–40 spectrofluorometer (excitation 493 nm, emission 530 nm) equipped with a thermostatized cuvette, stirring system and adding port. The assay solution contained 6 μM acridine orange, 250 mM sucrose, 100 mM *N*-methylglucamine gluconate and 10 mM Tris-Hepes (pH 7.5). After 2 ml of this buffer reached steady-state fluorescence (within 90 s) 50 μl of membrane vesicles (100–150 μg protein) preloaded with 250 mM sucrose, 100 mM *N*-methylglucamine gluconate, 10 mM Tris-Hepes (pH 6.0) was added. As previously described [29,31], there was a 30–40% quenching in the acridine orange fluorescence signal which reached equilibrium within 2 min. Sufficient quantities of sodium gluconate were then added with constant stirring to achieve a final concentration of 2.5 to 50 mM in the external buffer. The addition of Na^+ resulted in a collapse of the outwardly directed proton gradient and a reappearance of acridine orange fluorescence [28]. The increase in fluorescence after Na^+ addition was linear for more than 2 s, and the initial rate of acridine orange fluorescence recovery was measured as the initial slope [30]. After 300 s the pH gradient was dissipated with 50 mM potassium gluconate and 20 ng nigericin as described by Knickelbein et al. [32]. The small fluorescence quenching still remaining after nigericin addition was due to binding of the dye to the membrane [30]. Appropriate corrections were made for this binding of acridine orange to the membrane vesicles whenever the salt (*N*-methylglucamine gluconate) was replaced by mannitol, as described by Burnham et al. [33].

^{22}Na flux experiments. After preparation of the brush-border membrane vesicles as described above, the final pellet was resuspended and washed twice according to the method of Freiburg et al. [34]. All membrane preparations were initially washed in 300 mM mannitol, 5 mM Tris-Hepes (pH 7.5). In experiments where the pH of the intravesicular medium was pH 7.5, a second washing with 144 mM KCl, 5 mM Mes, 13 mM Tris, 13 mM Hepes (pH 7.5) was used and the final pellet brought up in the same buffer. In studies where the pH of the intravesicular medium was pH 5.5, the second washing solution contained 150 mM KCl, 25 mM Mes, 4.6 mM Tris (pH 5.5).

Uptake of ^{22}Na was measured at 20°C by a Millipore filtration technique as described by Murer et al. [35]. The incubation medium contained 144 mM KCl, 5 mM Mes, 13 mM Hepes, 13 mM Tris and 1 mM NaCl (pH 7.5). The experiment was started by the addition of 160 μl of the incubation media containing 1–2 μCi of ^{22}Na to 40 μl of the membrane suspension (100–150 μg protein). After a designated period of time, the reaction was terminated by the addition of 5 ml of ice-cold stop solution containing 150 mM LiCl, 16 mM Hepes, 10 mM Tris (pH 7.5). The diluted sample was immediately filtered through a 0.45 μm Millipore filter (HAWP), and the filter was washed three times with 5 ml of cold stopping solution. Filters were dissolved in scintillation fluid, and the radioactivity was measured in a Beckman LS-6800 scintillation counter. Each experiment was performed in triplicate on a

minimum of three separate membrane preparations, unless otherwise stated.

Stopped-flow nephelometric measurements

Measurements were performed with a home-made stopped-flow apparatus, the construction of which has been previously described [36]. The apparatus was made to function as a nephelometer by placing 500 nm filters (Omega Optical Co., Battleboro, VT) in the path of the excitation and emission (90° to incident) beam. One drive syringe of the stopped-flow device was loaded with a suspension of colonic membrane vesicles (0.2 mg protein per ml) in 100 mM mannitol/1.0 mM Tris-Hepes (pH 7.4). The other drive syringe was loaded with 450 mM mannitol/1 mM Tris-Hepes (pH 7.4). The two drive syringes were mixed in a one to one ratio by a plunger driven by compressed air. The intensity of 90° scattered light was measured by a photomultiplier tube and changes in scattered light intensity versus time were stored in digital form on a Digital PDP 11/10 computer (Digital Equipment Corporation, Maynard, MA) and plotted using a Hewlett-Packard 7015A *X-Y* recorder (Hewlett-Packard Company, Palo, Alto, CA). Exponential rate constants were calculated using a non-linear least-squares program. Temperature was maintained at 25°C with a circulating water bath.

Statistical methods

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

Materials

Unless otherwise indicated, all materials were obtained from Fisher Chemical Co. (Fairlawn, NJ) or Sigma Chemical Co. (St. Louis, MO).

Results

Fluorescence polarization studies

In agreement with prior studies [5,20,21], benzyl alcohol significantly increased the fluidity of rat colonic brush-border membranes, as assessed by steady-state fluorescence polarization using the probes diphenylhexatriene (DPH) and 12-AS (Table I). At concentrations of 25, 50 and 75 mM, benzyl alcohol incrased fluidity by approx. 8, 15 and 20%, respectively. As shown in Table I, however, methyl alcohol at concentrations up to 125 mM did not influence the fluidity of these membranes.

TABLE I

INFLUENCE OF BENZYL ALCOHOL AND METHYL ALCOHOL ON THE FLUIDITY OF RAT COLONIC BRUSH-BORDER MEMBRANES

Values presented are means ± S.E. of six separate experiments. Bzl. alc., benzyl alcohol; MeOH, methyl alcohol.

Probe	Preparation	Anisotropy (r) at 25°C	Limiting hindered anisotropy (r_∞) at 25°C	Order parameter (S) at 25°C
DPH	Control	0.236 ± 0.001	0.215 ± 0.002	0.767 ± 0.006
	25 mM Bzl. alc.	0.215 ± 0.001 *	0.187 ± 0.002 *	0.716 ± 0.005 *
	50 mM Bzl. alc.	0.200 ± 0.002 *	0.167 ± 0.002 *	0.676 ± 0.006 *
	75 mM Bzl. alc.	0.189 ± 0.002 *	0.152 ± 0.003 *	0.645 ± 0.007 *
	50 mM MeOH	0.236 ± 0.002	0.215 ± 0.003	0.767 ± 0.007
	125 mM MeOH	0.234 ± 0.002	0.212 ± 0.003	0.762 ± 0.006
12-AS	Control	0.102 ± 0.001	–	–
	25 mM Bzl. alc.	0.094 ± 0.001 *	–	–
	50 mM Bzl. alc.	0.087 ± 0.002 *	–	–
	75 mM Bzl. alc.	0.080 ± 0.002 *	–	–
	50 mM MeOH	0.102 ± 0.002	–	–
	125 MeOH	0.100 ± 0.001	–	–

* $P < 0.05$ or less compared to control values.

It should be noted that while the experiments with DPH and 12-AS produced similar results, the two probes differ in a number of respects [37–43]. Diphenylhexatriene molecules are rod-shaped [43], localize deep in the lipid bilayer [37] and are aligned relatively parallel to the phospholipid acyl chains [42,43]. The transition moments for absorption and emission of this probe are along its long axis [42]. Rotation along this axis will not result in depolarization, whereas, rotation about another axis normal to its long axis will cause depolarization [42]. The main depolarizing rotations sensed by diphenylhexatriene, therefore, are along its X and Z axes [42]. The anthroyloxy fatty acid probe 12-AS assumes a more spherical shape in bilayers than DPH [42], also localizes deep in the bilayer [38–42], and its emission moment is in the plane of the anthracene ring and at 30° to its short molecular axis. Thus the main depolarizing rotations of 12-AS are along its X and Y axes [42]. In biological and artifical membranes, the structural organization of the lipid bilayer appears to limit the extent of rotation of diphenylhexatriene; therefore, r_∞ values for this probe are high and largely determine r [25]. Other probes such as 12-AS yield relatively low values of r_∞ in bilayer membranes and their R values reflect mainly T_c, i.e., the speed of rotation [27,44].

In the present studies both the static and dynamic components of membrane lipid fluidity, as assessed by r_∞ and S of DPH and r values of 12-AS, respectively, were influenced by benzyl alcohol but not by methyl alcohol.

Na^+-H^+ exchange studies

Kinetics of the Na^+-H^+ exchange process were initially evaluated in brush-border membrane vesicle preparations by determining the effect of increasing sodium concentrations (2.5–50 mM) on pH-stimulated sodium influx using ^{22}Na uptake (Fig. 1) and on sodium-stimulated proton efflux with acridine orange fluorescence (Fig. 2) techniques. By both methods the exchange process demonstrated saturation kinetics, and at each concentration of sodium tested was inhibited by amiloride (1 mM) approximately 80% (not shown). As shown in Fig. 1, where substrate versus rate were plotted in double-reciprocal form [45] for the ^{22}Na uptake experiments, the results were linear

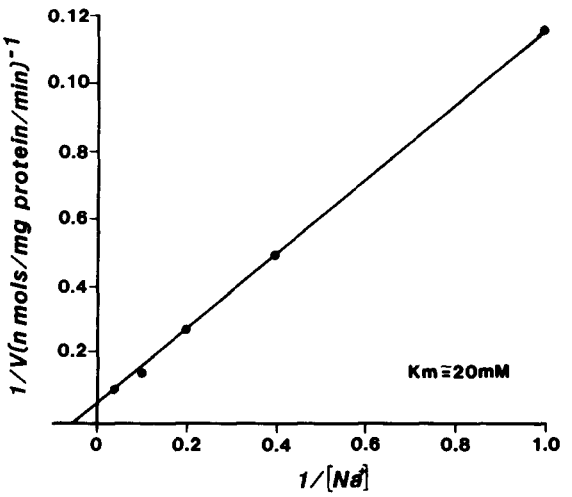

Fig. 1. pH-stimulated sodium influx was measured at 20°C in rat colonic brush-border membrane vesicles using ^{22}Na as described under Material and Methods. A representative double-reciprocal plot of proton efflux versus sodium concentrations is shown with a K_m for sodium of approx. 20 mM and a V_{max} of 17.1 nmol/mg protein per min.

and revealed a K_m for sodium of approx. 20.6 ± 1.1 mM and a maximum velocity (V_{max}) of 17.1 ± 1.5 nmol/mg protein per min ($N = 3$). Kinetic studies with acridine orange demonstrated a similar K_m for sodium of 24.0 ± 0.9 mM ($N = 3$), for these brush-border membrane vesicles under control conditions (Fig. 2). V_{max} values, however, using this fluorescent dye technique could only be expressed in arbitrary fluorescence units and, therefore, could not be directly compared to the values obtained for this kinetic parameter using ^{22}Na.

As assessed by percent increase in fluorescence units of acridine orange at 2 s, 50 mM and 75 mM benzyl but not methyl alcohol significantly increased sodium-stimulated proton efflux at all concentrations of sodium tested (2.5–50 mM) (not shown). The values for V_{max} and the K_m for sodium were then obtained from double-reciprocal plots (Fig. 2), and the data are summarized in Table II. Benzyl alcohol, at 50 and 75 mM concentrations, increased the V_{max} of this exchange process by approx. 58 and 75%, respectively. Neither concentration of this agent, however, altered the K_m for sodium. Similar concentrations of methyl alcohol did not significantly influence the kinetic parameters of this exchange process (Table II).

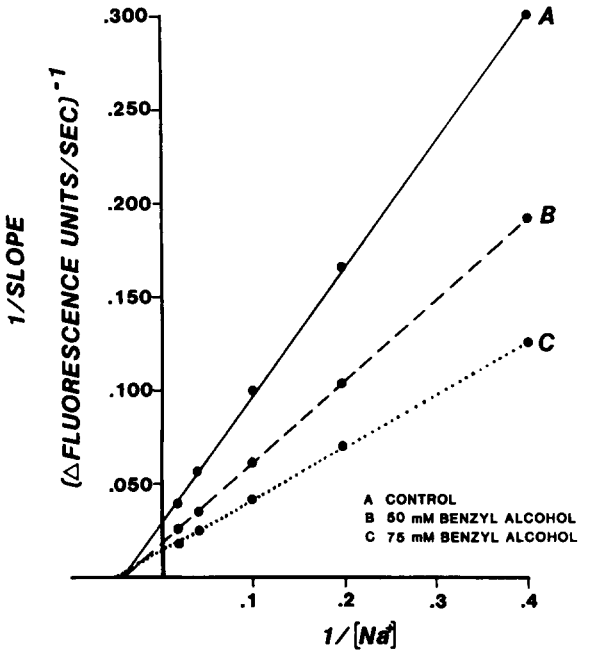

Fig. 2. Sodium-stimulated proton efflux was measured at 26°C in rat colonic brush-border membrane vesicles, using the pH-sensitive fluorescent dye, acridine orange, as described under Materials and Methods. Representative double-reciprocal plots of proton efflux versus sodium concentrations of three separate experiments under control conditions (A), and in the presence of concentrations of 50 mM benzyl alcohol (B) or 75 mM benzyl alcohol (C) are shown. Similar studies, using 50 and 75 mM concentrations of methyl alcohol, yielded plots which were superimposable on (A) and, therefore, are not shown (see Table II).

TABLE II

EFFECTS OF BENZYL ALCOHOL AND METHYL ALCOHOL ON THE KINETIC PARAMETERS OF SODIUM-STIMULATED PROTON EFFLUX IN RAT COLONIC BRUSH-BORDER MEMBRANE VESICLES

Values represent means ± S.E. of four separate preparations.

Preparation	V_{max} (arbitrary fluorescence) units	K_m of sodium (mM)
Control	36.6 ± 1.9	24.0 ± 0.9
50 mM benzyl alcohol	57.9 ± 4.8 *	24.1 ± 0.6
50 mM methyl alcohol	37.6 ± 3.6	23.8 ± 1.2
75 mM benzyl alcohol	74.0 ± 5.0 *	22.8 ± 1.4
75 mM methyl alcohol	36.8 ± 4.2	25.7 ± 1.9

* $P < 0.05$ compared to control values.

Osmotic water flow studies

Fig. 3 shows a representative plot of the intensity of scattered light versus time for rat colonic brush-border membranes exposed to a hyperosmotic gradient (175 mosM) in the presence of 75 mM benzyl or methyl alcohol. Scattered-light intensity rapidly increases after mixing which reflects the decrease in mean vesicular volume as osmotic water efflux occurs. The use of scattered light-intensity as an indicator of vesicular volume has been established in other membrane vesicular systems [16,19]. The increases in scattered-light intensity observed after mixing colonic membrane vesicles with hyperosmotic mannitol gradients could be fit to a single-exponential function, and the exponential rate constants reflect the osmotic permeability of the membranes [19]. The data for the rate constants of osmotic shrinkage seen in the presence of various concentrations of methyl or benzyl alcohol are given in Table III. Since the initial osmolalities, initial osmotic gradient, temperature, and other parameters were the same at a given alcohol concentration, the alterations in the rate constants of shrinkage could only reflect differences in the membrane osmotic permeability [19]. The rate constants in the presence of benzyl

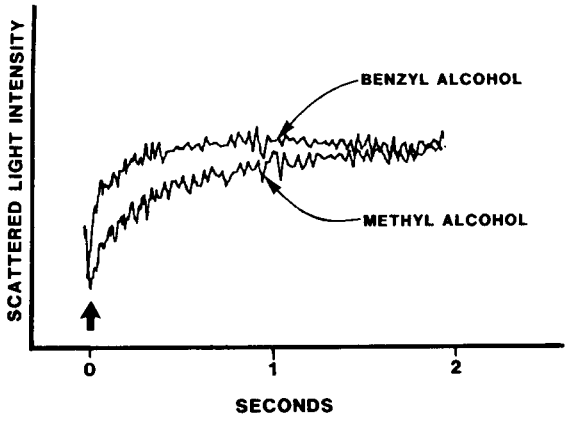

Fig. 3. Change in scattered light intensity versus time for colonic membrane vesicles exposed to a hyperosmotic mannitol solution in the presence of 75 mM benzyl or methyl alcohol. Mixing occurred at the arrow. Solutions were mixed in the stopped-flow apparatus as described in Materials and Methods; after mixing the initial intravesicular solution was 100 mM mannitol/1 mM Tris-Hepes and extravesicular solution was 275 mM mannitol/1 mM Tris-Hepes. Temperature was kept constant at 25°C.

TABLE III

RATES OF OSMOTIC WATER EFFLUX FROM RAT COLONIC BRUSH-BORDER MEMBRANE VESICLES EXPOSED TO VARIOUS CONCENTRATIONS OF METHYL OR BENZYL ALCOHOL

λ = rate constant for shrinkage. Values represent means $\pm$S.E. for eight separate determinations. Osmotic gradient was 175 mosM; temperature was 25°C.

Alcohol concn.	$\lambda(s^{-1})$	
	methyl alcohol	benzyl alcohol
25 mM	2.24 ± 0.09	3.23 ± 0.49
50 mM	2.68 ± 0.24	3.88 ± 0.53
75 mM	2.70 ± 0.21	4.69 ± 0.27 *

* $P < 0.05$ compared to methyl alcohol value.

alcohol were greater than those in the presence of methyl alcohol at each concentration, and the difference was statistically significant ($P < 0.05$) at 75 mM alcohol. This observation suggests a significant increase in membrane osmotic permeability at 75 mM benzyl alcohol.

Discussion

Anesthetic alcohols such as benzyl alcohol are known to decrease the lipid order, i.e., increase the fluidity of model and biological membranes [5,20,21]. The ability of alcohols to decrease the lipid order of membranes has previously been shown to correlate well with their lipid : water partition coefficients [46], hence explaining the lack of an effect of methyl alcohol on fluidity at the concentrations used in the present experiments.

Prior studies in our laboratory [5] and others [21] have demonstrated that the increased fluidity of plasma membranes induced by benzyl alcohol could affect the activities of several membrane-bound enzyme activities. Certain *n*-aliphatic alcohols have also been shown to modulate Na^+-coupled D-glucose uptake by altering the fluidity of rat small intestinal brush-border membranes [46]. In agreement with these studies, the present data strongly suggest that as the lipid order of rat colonic brush-border membrane vesicles is decreased by benzyl alcohol, osmotic water permeability and sodium-stimulated proton efflux are increased.

It has previously been demonstrated that cholesterol significantly decreased or increased the water permeability of planar lipid bilayers [17] and vesicular bilayer liposomes [16,18], when the membranes were above or below their phase transition temperatures, respectively. The effects on water permeability correlate with the effects of cholesterol on increasing lipid order above the phase transition temperature and decreasing it below this temperature [47]. In liposomes, an increase in the ratio of unsaturated to saturated fatty acyl side chains also has been shown to enhance water permeability [16]. An increase in this ratio would theoretically result in an increased fluidity [48,49] and would be consistent with the effects of cholesterol on water permeability and lipid order.

The pathways for osmotic water flow across the colonic epithelium are not well defined. Whether transepithelial water flow is primarily paracellular or transcellular remains unclear. If water flow occurs by a transcellular pathway, water must cross epithelial membranes via transmembrane channels or by dissolving in the lipids of the membrane and diffusing across it. Water flow across red cell membranes is thought to occur mainly through aqueous pores [50]. In these cells, treatment with anesthetic *n*-alkanols decreases lipid order but also slightly decreases water permeability [51]. Since water bypasses the lipid domains by flow through the aqueous transmembrane protein pores, it would appear that the physical state of the membrane lipids does not greatly affect water permeability. The inhibition of water permeability in red cells by *n*-alkanols may actually be due to denaturation of the protein pores, similar to the effect seen on water permeability with sulfhydryl-reactive agents [50].

In contrast, recent studies in rat small intestinal brush-border membrane vesicles suggest that water flow across these membranes occurs predominantly by diffusion across the lipid bilayer and not through aqueous pores [19]. While speculative, the present data demonstrating the influence of lipid fluidity on osmotic flow would suggest that this may also be true in the case of rat colonic brush-border membranes. Although the presence of aqueous pores cannot be excluded, our results suggest that the major transcellular pathway for water flow in these membranes is by the 'solubil-

ity-diffusion' mechanism which has been proposed for model lipid bilayers [52].

Recently, our laboratory has demonstrated that increased phospholipid methylation in rat colonic brush-border membranes can increase the lipid fluidity of these membranes [53] as well as enhance amiloride-sensitive sodium-stimulated proton efflux across brush-border vesicles [54]. Changes in fluidity of approx. 5–6% in these studies were associated with a 36% increase in the V_{max} of this process. In the present results, increases of fluidity with 25 mM and 50 mM benzyl alcohol were about 9% and 15%, respectively. These changes increased the V_{max} of Na^+-H^+ exchange by approx. 58% and 75%, respectively. Therefore, based on these observations, fluidity per se may modulate the Na^+-H^+ exchange process in rat colonic brush-border membranes. Such changes in Na^+-H^+ exchange would be important for electroneutral sodium absorption in the rat colon.

Additionally, recent studies [28,29,32,55–57] have suggested that Na^+/H^+ exchangers in plasma membranes of various cell types may play important roles in a number of diverse physiological functions including initiation of proliferation [57]. Thus, the ability of fluidity to modulate this process in rat colonic brush-border membranes may have additional physiological significance.

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