Modulation of lipid fluidity of small- and large-intestinal antipodal membranes by Ca^{2+}

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A series of experiments were conducted to examine the role of Ca^{2+} in modulating the fluidity of rat small- and large-intestinal antipodal plasma membranes and their liposomes. This bivalent cation was found to decrease the fluidity of these preparations in a complex manner involving at least two distinct mechanisms. The first appeared to be a direct effect of Ca^{2+} on fluidity, was readily reversible by addition of EGTA and presumably involved binding of Ca^{2+} to anionic sites in the lipid bilayers of these membranes. This effect was seen with all preparations examined. In contrast, the second effect of Ca^{2+} on fluidity was only seen in intact small-intestinal brush-border membranes, appeared to be indirect, was time- and cation-dependent, was only minimally reversible by addition of EGTA, and appeared to involve stimulation of membrane-bound enzymes which altered this membrane's fatty acid composition. Furthermore, regional differences in this latter effect of Ca^{2+} on brush-border membrane fluidity were also seen in these studies, i.e. proximal > distal small intestine.

INTRODUCTION

The luminal (brush border) and contraluminal (basolateral) plasma membranes of enterocytes and colonocytes, the predominant cells lining the small and large intestine respectively, are highly specialized to perform certain physiological functions (Douglas *et al.*, 1972; Brasitus & Keresztes, 1983, 1984). These antipodal membranes in both cell types, which function to regulate exchange between organism and environment, also differ from each other in a number of respects (Bloom & Fawcett, 1968; Forstner *et al.*, 1968; Douglas *et al.*, 1972), including lipid composition and fluidity (Forstner *et al.*, 1968; Brasitus & Schachter, 1980a; Brasitus *et al.*, 1980; Brasitus & Keresztes, 1983, 1984). Moreover, considerable evidence exists that alterations in the lipid fluidity of these membranes can influence many important functions of these cells, including the activities of certain enzymes such as alkaline phosphatase (Brasitus *et al.*, 1979), adenylate cyclase (Brasitus & Schachter, 1980a) and guanylate cyclase (Brasitus & Schachter, 1980b), Na^+ + K^+ -dependent ATPase (Brasitus & Schachter, 1980a) and Ca^{2+} -dependent ATPase (Brasitus *et al.*, 1979), as well as D-glucose transport (Brasitus *et al.*, 1979), Na^+ /H $^+$ exchange (Brasitus *et al.*, 1986) and water permeability (Brasitus *et al.*, 1986).

The mechanisms which regulate the lipid fluidity of these intestinal antipodal membranes, however, are relatively undefined. Accordingly, the present studies were initiated to examine the role of Ca^{2+} in the regulation of lipid fluidity of these various intestinal plasma membranes. Several lines of evidence point to this possibility. Ca^{2+} ions have been shown to interact with phospholipid monolayers (Shah & Schulman, 1967) and model bilayers (Ohnishi & Ito, 1974) to decrease their

lipid fluidity. This bivalent cation has also been shown to cause similar fluidity changes in a number of biological membranes, including pig (Ohyashiki & Mohri, 1982) and rat (Schachter & Shinitzky, 1977) small-intestinal brush-border membranes. In this regard, previous studies in hepatocytes have suggested that fluidity alterations induced by Ca^{2+} may be more complex than was previously appreciated (Livingstone & Schachter, 1980) and may be restricted to certain regions of these cells' plasma membranes (Storch *et al.*, 1983). The results described below demonstrate that Ca^{2+} can interact with intestinal antipodal plasma membranes in a complex manner involving both 'reversible' and 'irreversible' mechanisms for altering lipid fluidity. The former mode of action of Ca^{2+} on fluidity appears to involve binding of the ions to anionic sites in the lipid bilayer, and can be detected in all intestinal antipodal membranes examined. In contrast, the latter effect of Ca^{2+} on fluidity appears to involve stimulation of certain membrane-bound enzymes which alter membrane fatty acid composition and is restricted to small-intestinal brush-border membranes. These results, as well as discussion of their possible significance, serve as the basis for the present paper.

EXPERIMENTAL

Materials

Fatty acid, methyl esters, g.l.c. columns and lipid standards were all purchased from Applied Science Corp. (State College, PA, U.S.A.) and/or Supelco (Bellefonte, PA, U.S.A.). All other materials were obtained from either Fisher Chemical Co. (Fair Lawn, NJ, U.S.A.) or Sigma Chemical Co. (St. Louis, MO, U.S.A.), unless otherwise indicated.

Abbreviation used: DPH, 1,6-diphenylhexa-1,3,5-triene.

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Membrane preparations

Male albino rats of the Sherman strain, weighing 250–300 g, were used for all studies. The animals were starved for 18 h, with water *ad libitum*, before being killed. They were killed rapidly by cervical dislocation and their proximal and/or distal small intestines and colons, minus the caecum, were excised. Brush-border membranes were prepared from the proximal and distal halves of the small intestine, by using 10 mM-MgCl₂ instead of 10 mM-CaCl₂ as the precipitating agent (Brasitus *et al.*, 1979), and colon (Brasitus & Keresztes, 1984) as described previously. Basolateral membranes from the proximal half of the small intestine (Brasitus *et al.*, 1980) and colon (Brasitus & Keresztes, 1983) were also prepared as described previously. Purity and comparability of the preparations as well as contamination by microsomal and mitochondrial membranes were assessed by using appropriate marker enzymes (Brasitus *et al.*, 1979; Brasitus & Schachter, 1980a; Brasitus & Keresztes, 1984). All plasma membranes were purified 15–20-fold compared with the original homogenates and showed minimal contamination by intracellular membranes, as described previously (Brasitus *et al.*, 1979; Brasitus & Schachter, 1980a; Brasitus & Keresztes, 1984). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Liposomes

Liposomes were prepared from extracted plasma-membrane lipid as previously described (Brasitus *et al.*, 1979). Briefly, lipid was extracted from these membranes as described by Folch *et al.* (1957), and approx. 0.3–0.5 mg of dried lipid was suspended in phosphate-buffered saline (Brasitus *et al.*, 1979). The suspension was then sonicated under N₂ at 4 °C for 7–10 min. Thereafter, the preparations were centrifuged at 10000 *g* for 10 min and the resulting supernatant was used for fluorescence-polarization studies (see below).

Chemical determinations

The composition of the lipid extracts was examined by t.l.c. as described by Katz *et al.* (1976). Total phospholipid was measured by the method of Ames & Dubin (1960), and cholesterol by the procedure of Zlatkis *et al.* (1953). Derivatives of fatty acids of the total lipid extract were prepared by the procedure described by Gartner & Vahouny (1972). Fatty acid methyl esters were analysed on a Hewlett–Packard 5790A gas–liquid chromatograph equipped with a flame ionization detector and interfaced with a Hewlett–Packard 3390A integrator, authentic fatty acid methyl esters being used to identify retention times (Gartner & Vahouny, 1972).

Fluorescence-polarization studies

Lipid fluidity was assessed by the steady-state fluorescence polarization of 1,6-diphenylhexa-1,3,5-triene (DPH) (Aldrich Chemical Co., Milwaukee, WI, U.S.A.). Steady-state fluorescence measurements were made with a Perkin–Elmer 650–40 fluorescence spectrofluorimeter adapted for fluorescence polarization. The methods used to load the membranes and liposomes and the quantification of the polarization of fluorescence have been described previously (Brasitus & Schachter, 1982; Brasitus & Dudeja, 1985). The content of the fluorophore DPH in the preparations was measured fluorimetrically as described by Cogan & Schachter (1981). Final molar

ratios of probe/lipid ranged from 0.001 to 0.003, and the anisotropy differences noted in these studies could not be ascribed to differences in DPH concentrations in the membranes. Corrections for light-scattering (suspensions minus probe) and for fluorescence in the ambient medium (determined by pelleting the preparations after each measurement) were made routinely, and the combined corrections were less than 3% of the total fluorescence intensity observed for DPH-loaded preparations.

Fluorescence polarization was expressed as the fluorescence anisotropy, r (Brasitus & Schachter, 1980a). The results were obtained according to the modified Perrin relationship (Heyn, 1979; Jahnig, 1979):

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

where r is the fluorescence anisotropy, r_0 is the maximal limiting anisotropy, taken as 0.365 for DPH (Shinitzky & Barenholz, 1974), r_{∞} is the limiting hindered anisotropy, T_c is the correlation time, and T_f is the mean lifetime of the excited state. Values of r_{∞} for DPH were calculated from r values as described by Van Blitterswijk *et al.* (1981). The ‘order parameter’ component of membrane fluidity was assessed by S , where $S = (r_{\infty}/r_0)^{1/2}$, as described previously (Van Blitterswijk *et al.*, 1981). No change in the excited-state lifetimes, as assessed by total fluorescence intensity, was demonstrated for DPH in the present studies.

To investigate the effects of Ca²⁺, membrane suspensions were incubated with DPH in buffer containing 5 mM-Tris/HCl, 146 mM-NaCl, 4 mM-KCl, 0.1 mM-EGTA, pH 7.4, and 0–4 mM-CaCl₂ as required. After incubation at 37 °C for 2 h, EGTA (pH 7.4) was added to certain samples (see below) to a final concentration of 8 mM before fluorescence-polarization studies (Livingstone & Schachter, 1980). For time-course studies of Ca²⁺ effects, 8 mM-EGTA was added at different time intervals as required (Livingstone & Schachter, 1980).

Statistical methods

All results are expressed as means ± S.E.M. Paired or unpaired *t* tests were used for all statistical analysis: $P < 0.05$ was considered significant.

RESULTS

Effects of Ca²⁺ concentration on plasma-membrane fluidity

To examine the effects of Ca²⁺ on membrane fluidity, plasma membranes prepared from the small and large intestine and their liposomes were loaded with DPH and simultaneously incubated with various concentrations of Ca²⁺ (0–4 mM) at 37 °C for 2 h. The r , r_{∞} and S values of DPH for each of these preparations were then measured (Tables 1 and 2). As shown in these Tables, Ca²⁺ significantly decreased the fluidity of each of these preparations.

Previous studies in intact hepatocyte plasma membranes had indicated that this phenomenon was largely irreversible (Livingstone & Schachter, 1980). To examine the reversibility of the Ca²⁺ effect on these preparations, each was incubated with DPH and various concentrations of Ca²⁺ (0–4 mM) and, thereafter, EGTA (final concn. 8 mM) was added, the suspensions were incubated at 37 °C for an additional 30 min, and DPH fluorescence values were measured (Tables 3 and 4). As shown in these Tables, with the exception of small-intestinal brush-

Table 1. Fluorescence-polarization studies using DPH on the effects of Ca²⁺ on small-intestinal plasma membranes and liposomes

Values represent means $\pm$ S.E.M. for the numbers of preparations (*n*) determined at 25 °C. Abbreviations: (P), proximal; (D), distal; B.B.M., brush-border membrane; B.L.M., basolateral membrane. **P* < 0.05 or less compared with 'No addition' values of similar preparations.

Preparation	Condition	(<i>n</i>)	Anisotropy (<i>r</i>)	Mean limiting hindered anisotropy (<i>r</i> _∞)	Mean order parameter (<i>S</i>)
(P) B.B.M.	No addition	4	0.269 $\pm$ 0.002	0.259	0.842
(P) Liposomes	No addition	4	0.200 $\pm$ 0.002	0.167	0.675
(D) B.B.M.	No addition	4	0.276 $\pm$ 0.002	0.268	0.857
(D) Liposomes	No addition	4	0.208 $\pm$ 0.002	0.177	0.696
(P) B.B.M.	+ 1 mM-CaCl ₂	4	0.275 $\pm$ 0.001*	0.266	0.854
(D) B.B.M.	+ 1 mM-CaCl ₂	4	0.283 $\pm$ 0.002*	0.277	0.871
(P) B.B.M.	+ 2 mM-CaCl ₂	4	0.285 $\pm$ 0.001*	0.280	0.876
(D) B.B.M.	+ 2 mM-CaCl ₂	4	0.291 $\pm$ 0.001*	0.288	0.888
(P) B.B.M.	+ 4 mM-CaCl ₂	4	0.285 $\pm$ 0.001*	0.280	0.876
(P) Liposomes	+ 4 mM-CaCl ₂	4	0.208 $\pm$ 0.002*	0.177	0.696
(D) B.B.M.	+ 4 mM-CaCl ₂	4	0.291 $\pm$ 0.001*	0.288	0.888
(D) Liposomes	+ 4 mM-CaCl ₂	4	0.215 $\pm$ 0.002*	0.187	0.716
(P) B.L.M.	No addition	6	0.246 $\pm$ 0.003	0.228	0.790
(P) Liposomes	No addition	4	0.173 $\pm$ 0.002	0.131	0.599
(P) B.L.M.	+ 1 mM-CaCl ₂	6	0.247 $\pm$ 0.002	0.229	0.792
(P) B.L.M.	+ 2 mM-CaCl ₂	6	0.252 $\pm$ 0.002*	0.226	0.787
(P) B.L.M.	+ 4 mM-CaCl ₂	6	0.252 $\pm$ 0.002*	0.226	0.787
(P) Liposomes	+ 4 mM-CaCl ₂	4	0.182 $\pm$ 0.002*	0.143	0.626

Table 2. Fluorescence-polarization studies using DPH on the effects of Ca²⁺ on colonic plasma membranes and liposomes

Values represent means $\pm$ S.E.M. for the numbers of preparations (*n*) determined at 25 °C. Abbreviations: B.B.M., brush-border membrane; B.L.M., basolateral membrane. **P* < 0.05 or less compared with 'No addition' values of similar preparations.

Preparation	Condition	(<i>n</i>)	Anisotropy (<i>r</i>)	Mean limiting hindered anisotropy (<i>r</i> _∞)	Mean order parameter (<i>S</i>)
B.B.M.	No addition	9	0.237 $\pm$ 0.001	0.216	0.769
Liposomes	No addition	4	0.164 $\pm$ 0.002	0.119	0.571
B.B.M.	+ 1 mM-CaCl ₂	9	0.240 $\pm$ 0.001	0.220	0.776
B.B.M.	+ 2 mM-CaCl ₂	7	0.242 $\pm$ 0.001*	0.223	0.782
B.B.M.	+ 4 mM-CaCl ₂	9	0.244 $\pm$ 0.001*	0.225	0.785
Liposomes	+ 4 mM-CaCl ₂	4	0.173 $\pm$ 0.002*	0.131	0.599
B.L.M.	No addition	4	0.201 $\pm$ 0.002	0.168	0.678
Liposomes	No addition	4	0.142 $\pm$ 0.002	0.089	0.494
B.L.M.	+ 1 mM-CaCl ₂	4	0.206 $\pm$ 0.001*	0.175	0.692
B.L.M.	+ 2 mM-CaCl ₂	4	0.209 $\pm$ 0.001*	0.179	0.700
B.L.M.	+ 4 mM-CaCl ₂	4	0.211 $\pm$ 0.001*	0.181	0.704
Liposomes	+ 4 mM-CaCl ₂	4	0.154 $\pm$ 0.002*	0.105	0.536

border membranes, the addition of EGTA completely reversed these Ca²⁺-induced changes in membrane fluidity. Furthermore, as shown in Table 3, this 'irreversible' effect of Ca²⁺ on fluidity was greater in brush-border membranes prepared from proximal than from distal small intestine. To ensure that the EGTA completely removed Ca²⁺ from these plasma membranes, tracer amounts of ⁴⁵CaCl₂ were added with non-radioactive Ca²⁺ to the initial incubation mixtures (Livingstone & Schachter, 1980). Less than 0.3% of the quantity initially added to the tracer was found in the membranes after treatment with 8 mM-EGTA and

harvesting of membranes by centrifugation (results not shown).

From the present observations (Tables 1–4), and in agreement with previous studies in rat hepatocyte membranes (Livingstone & Schachter, 1980), Ca²⁺ appears to have at least two effects on the fluidity of these preparations, i.e. an 'irreversible' and a 'reversible' effect. The 'irreversible' effect was seen only in intact small-intestinal brush-border membranes (proximal > distal), whereas the 'reversible' effect was shown to occur in all other preparations, including liposomes prepared from small-intestinal brush-border membranes. As

Table 3. Fluorescence-polarization studies using DPH on the effects of Ca^{2+} on small-intestinal plasma membranes and liposomes after treatment with EGTA

Values represent means $\pm$ S.E.M. for the numbers of preparations (n) determined at 25 °C. Abbreviations: (P), proximal; (D), distal; B.B.M., brush-border membrane; B.L.M., basolateral membrane. * $P < 0.05$ or less compared with 'No addition' values of similar preparations.

Preparation	Condition	(n)	Anisotropy (r)	Mean limiting hindered anisotropy (r_∞)	Mean order parameter (S)
(P) B.B.M.	No addition	4	0.267 ± 0.002	0.256	0.837
(P) Liposomes	No addition	3	0.201 ± 0.002	0.168	0.678
(D) B.B.M.	No addition	4	0.275 ± 0.002	0.267	0.855
(D) Liposomes	No addition	3	0.207 ± 0.002	0.176	0.694
(P) B.B.M.	+ 1 mM- CaCl_2	4	$0.273 \pm 0.001^*$	0.264	0.850
(D) B.B.M.	+ 1 mM- CaCl_2	4	$0.279 \pm 0.001^*$	0.272	0.863
(P) B.B.M.	+ 2 mM- CaCl_2	4	$0.283 \pm 0.002^*$	0.277	0.871
(D) B.B.M.	+ 2 mM- CaCl_2	4	$0.281 \pm 0.002^*$	0.275	0.868
(P) B.B.M.	+ 4 mM- CaCl_2	4	$0.285 \pm 0.004^*$	0.280	0.876
(P) Liposomes	+ 4 mM- CaCl_2	3	0.203 ± 0.003	0.171	0.684
(D) B.B.M.	+ 4 mM- CaCl_2	4	$0.283 \pm 0.002^*$	0.277	0.871
(D) Liposomes	+ 4 mM- CaCl_2	3	0.207 ± 0.002	0.176	0.694
(P) B.L.M.	No addition	6	0.223 ± 0.004	0.197	0.735
(P) Liposomes	No addition	3	0.175 ± 0.002	0.133	0.604
(P) B.L.M.	+ 1 mM- CaCl_2	6	0.225 ± 0.004	0.200	0.740
(P) B.L.M.	+ 2 mM- CaCl_2	6	0.228 ± 0.004	0.204	0.748
(P) B.L.M.	+ 4 mM- CaCl_2	6	0.231 ± 0.005	0.208	0.755
(P) Liposomes	+ 4 mM- CaCl_2	3	0.176 ± 0.003	0.135	0.608

Table 4. Fluorescence-polarization studies using DPH on the effects of Ca^{2+} on colonic plasma membranes and liposomes after treatment with EGTA

Values represent means $\pm$ S.E.M. for the numbers of preparations (n) determined at 25 °C. Abbreviations: B.B.M., brush-border membrane; B.L.M., basolateral membrane. * $P < 0.05$ or less compared with 'No addition' values of similar preparations.

Preparation	Condition	(n)	Anisotropy (r)	Mean limiting hindered anisotropy (r_∞)	Mean order parameter (S)
B.B.M.	No addition	9	0.239 ± 0.002	0.219	0.775
Liposomes	No addition	3	0.165 ± 0.003	0.120	0.573
B.B.M.	+ 1 mM- CaCl_2	9	0.242 ± 0.003	0.223	0.782
B.B.M.	+ 2 mM- CaCl_2	7	0.243 ± 0.003	0.224	0.783
B.B.M.	+ 4 mM- CaCl_2	9	0.243 ± 0.003	0.224	0.783
Liposomes	+ 4 mM- CaCl_2	3	0.167 ± 0.004	0.123	0.580
B.L.M.	No addition	4	0.200 ± 0.003	0.167	0.676
Liposomes	No addition	3	0.144 ± 0.003	0.092	0.502
B.L.M.	+ 1 mM- CaCl_2	4	0.201 ± 0.003	0.168	0.678
B.L.M.	+ 2 mM- CaCl_2	4	0.199 ± 0.004	0.165	0.672
B.L.M.	+ 4 mM- CaCl_2	4	0.203 ± 0.004	0.171	0.684
Liposomes	+ 4 mM- CaCl_2	3	0.145 ± 0.002	0.093	0.505

shown in Fig. 1, the apparent half-saturation concentration for this 'irreversible' effect of Ca^{2+} on fluidity of rat proximal small-intestinal brush-border membranes was approx. 1.25 mM.

Time course of Ca^{2+} effects on membrane fluidity

The time course of the 'irreversible' effect of Ca^{2+} on fluidity of proximal small-intestinal brush-border membranes was assessed in the following two ways: (1) membranes were incubated with DPH and 4 mM- Ca^{2+} at 37 °C, and at various times (5–120 min) EGTA was added and r values were determined; and (2) membranes were incubated as described above, and after EGTA

treatment liposomes were prepared from the lipids of these membranes and r values estimated. As shown in Fig. 2, addition of 4 mM- Ca^{2+} to the intact membranes and liposomes prepared from these membranes increased the r values of DPH of both of these preparations rapidly, with approx. 55% of the total increment by 15 min.

Specificity of bivalent-cation effect on membrane fluidity

To assess the relative specificity of the effects of Ca^{2+} on fluidity of rat proximal small-intestinal brush-border membranes, various bivalent cations (final concn. 4 mM)

Fig. 1. Effects of various Ca²⁺ concentrations on the fluorescence anisotropy (*r*) of DPH in small-intestinal brush-border membranes

Values are means $\pm$ S.E.M. for three membrane preparations. Membrane suspensions were incubated with Ca²⁺ (1–4 mM) for 2 h at 37 °C. The *r* values were measured at 25 °C. The percentage increases in *r* values of DPH with respect to the values observed in the absence of Ca²⁺ (control) are plotted.

Fig. 2. Time course of Ca²⁺ effect on DPH *r* values in intestinal brush-border membranes (●) and liposomes (○) prepared from membranes preincubated with 4 mM-Ca²⁺ at 37 °C

The *r* values were measured at 25 °C. Each data point in the Figure is a mean value for three preparations of membranes and liposomes. S.E.M. values are omitted for clarity, since all were less than 4% of the mean values.

were incubated at 37 °C for 2 h with intact membranes. The *r* values of DPH were then measured and, thereafter, portions of each sample were incubated with 8 mM-EGTA for an additional 30 min and *r* values were again determined.

As shown in Table 5, Ca²⁺, Mn²⁺ and Zn²⁺ appeared to increase *r* values significantly over control values. After incubation with EGTA, however, only Ca²⁺-treated samples retained their increment in *r* values over control values. In fact, Mn²⁺- and Zn²⁺-treated membranes actually became more fluid than their control counterparts

Table 5. Effects of various bivalent cations on rat proximal small-intestinal brush-border membrane fluidity studied with DPH

Values represent means $\pm$ S.E.M. for the numbers of preparations (*n*): * *P* < 0.05 or less compared with control value.

Addition	<i>(n)</i>	Anisotropy (<i>r</i>) at 25 °C	
		Before EGTA	After EGTA
None	6	0.266 $\pm$ 0.002	0.270 $\pm$ 0.003
4 mM-Ca ²⁺	5	0.286 $\pm$ 0.004*	0.283 $\pm$ 0.003*
4 mM-Zn ²⁺	6	0.274 $\pm$ 0.002*	0.255 $\pm$ 0.003*
4 mM-Mn ²⁺	6	0.286 $\pm$ 0.002*	0.261 $\pm$ 0.004*
4 mM-Mg ²⁺	6	0.270 $\pm$ 0.004	0.275 $\pm$ 0.003
4 mM-Ba ²⁺	6	0.271 $\pm$ 0.003	0.272 $\pm$ 0.003
4 mM-Sr ²⁺	6	0.271 $\pm$ 0.003	0.271 $\pm$ 0.004

(Table 5). The mechanism(s) responsible for the latter changes are unclear at present and will require further examination. In agreement with previous studies using hepatic plasma membranes (Livingstone & Schachter, 1980), however, the increase in *r* values, not reversible by EGTA, was highly specific for Ca²⁺ as compared with other bivalent cations.

Effect of Ca²⁺ treatment on membrane lipid composition

In the present experiments, rat proximal small-intestinal brush-border membranes treated with various concentrations (1–4 mM) of Ca²⁺ were found to possess a lower fluidity than their control counterparts. In this regard, previous studies in the model bilayers and natural membranes have correlated a low lipid fluidity with high molar ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine, as well as more saturated or longer acyl chains in phospholipids (Hubbell & McConnell, 1971; Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978). It was therefore decided to examine and compare the lipid composition as well as these parameters in control and Ca²⁺-treated (4 mM) membranes. As shown in Table 6, control and Ca²⁺-treated membranes possessed similar molar ratios of cholesterol/phospholipids and sphingomyelin/phosphatidylcholine. Ca²⁺-treated membranes, however, possessed a signifi-

Table 6. Compositional analysis of lipid extracts of rat proximal small-intestinal brush-border membranes treated with 4 mM-CaCl₂

Values are means $\pm$ S.E.M. for 16 determinations on five separate preparations of each membrane: * *P* < 0.05 or less compared with control value. 'Saturation index' is calculated by dividing the total of saturated acyl chains by the sum of each unsaturated chain multiplied by the number of double bonds.

Parameter	Control	Ca ²⁺ (4 mM)
Cholesterol/phospholipid (mol/mol)	1.06 $\pm$ 0.03	1.11 $\pm$ 0.03
Sphingomyelin/phosphatidylcholine (mol/mol)	0.36 $\pm$ 0.02	0.38 $\pm$ 0.02
Saturation index	0.62 $\pm$ 0.06	0.84 $\pm$ 0.06*

Table 7. Compositional analysis of total fatty acids of rat proximal small-intestinal brush-border membranes treated with 4 mM-CaCl₂

Values are means $\pm$ S.E.M. for 16 determinations on five separate preparations of each membrane; * $P < 0.05$ or less compared with control values.

Fatty acids	Composition (% , by mass)	
	Control	Ca ²⁺ (4 mM)
C _{14:0}	0.35 $\pm$ 0.05	0.49 $\pm$ 0.09
C _{14:1}	0.32 $\pm$ 0.03	0.45 $\pm$ 0.16
C _{16:0}	25.70 $\pm$ 0.59	27.51 $\pm$ 0.89
C _{16:1}	1.53 $\pm$ 0.13	1.21 $\pm$ 0.19
C _{18:0}	31.70 $\pm$ 1.18	36.40 $\pm$ 2.08*
C _{18:1}	13.60 $\pm$ 0.57	14.20 $\pm$ 0.52
C _{18:2}	16.60 $\pm$ 1.06	12.60 $\pm$ 0.55*
C _{20:4}	11.10 $\pm$ 0.59	8.90 $\pm$ 0.97*

cantly higher saturation index than their control counterparts. Furthermore, analyses of the total fatty acids of these membranes (Table 7) demonstrated that Ca²⁺-treated preparations possessed a significantly greater percentage of stearic acid (C_{18:0}) and lower percentages of linoleic acid (C_{18:2}) and arachidonic acid (C_{20:4}) than did untreated membranes. These differences in fatty acids appeared to be responsible for the higher saturation index seen in Ca²⁺-treated membranes and appeared to explain, at least in part, the differences in fluidity present between control and Ca²⁺-treated membranes (Shinitzky & Barenholz, 1978).

DISCUSSION

The present results demonstrate that Ca²⁺ can decrease the fluidity of antipodal membranes prepared from the small and large intestine of the rat. In agreement with previous studies in rat hepatocytes (Livingstone & Schachter, 1980), moreover, this effect of Ca²⁺ appeared to be complex and involved at least two different mechanisms. The first appeared to be a direct effect of this bivalent cation on fluidity, was readily reversed by addition of EGTA to chelate Ca²⁺ and presumably involved binding of Ca²⁺ to anionic sites such as acidic phospholipid head groups and sialic acid residues in the lipid bilayers of these membranes. This effect was seen with all preparations examined, and therefore seemed to be a generalized phenomenon.

The second effect of Ca²⁺ on fluidity was only seen in intact small-intestinal brush-border membranes. This effect appeared to be indirect, was time- and cation-concentration-dependent, was only minimally reversible (approx. 25%) by addition of EGTA, and appeared to involve membrane-bound enzymes which altered this membrane's fatty acid composition. Furthermore, regional differences in the latter effect of Ca²⁺ on brush-border membrane fluidity were also seen in these studies, i.e. proximal > distal small intestine.

It should be noted that in the present studies fluidity was assessed by steady-state fluorescence-polarization techniques with the probe DPH. It has been shown previously that, in biological and artificial membranes, the structural organization of the bilayer appears to limit

the rotation of DPH; therefore r_{∞} values for DPH are high, and largely determine r (Chen *et al.*, 1977; Van Blitterswijk *et al.*, 1981). As a result, this probe mainly assesses the 'order parameter' of fluidity (Schachter, 1984). In contrast, other probes such as the anthroyloxy derivatives yield relatively low values of r_{∞} in bilayer membranes, and their r values reflect mainly T_c , i.e. the speed of rotation (Schachter *et al.*, 1982; Vincent *et al.*, 1982). The latter probes therefore assess mainly the 'dynamic' component of fluidity (Schachter, 1984). In the present studies, attempts were made to use DL-2-(9-anthroyl)stearic acid and DL-12-(9-anthroyl)stearic acid to assess the influence of Ca²⁺ on fluidity. The results of these experiments, however, could not be interpreted, since Ca²⁺ was found to alter the lifetimes of the excited state of these probes. Therefore it is important to emphasize that the present data deal mainly with the effects of Ca²⁺ on the 'order parameter' of fluidity.

The exact mechanisms whereby Ca²⁺ treatment produced alterations in fatty acid composition and degree of saturation, which in turn decreased fluidity of these plasma membranes, are at present unclear. Previous studies, however, have shown that certain desaturase activities in microsomal membranes of hepatocytes could alter plasma-membrane fluidity (Storch & Schachter, 1984). In this regard, our laboratory has described such desaturase activities in rat enterocytes and colonocytes (Ehrlich *et al.*, 1985). However, these activities were not found to be present in purified plasma-membrane fractions, but only in microsomal membranes of these cells (Ehrlich *et al.*, 1985; J. B. Ehrlich, P. K. Dahiya & T. A. Brasitus, unpublished work). Since intact brush-border membranes were utilized in the present studies and were found to be minimally contaminated with intracellular membranes, the possibility that Ca²⁺ influenced fluidity via alterations in desaturase activities is unlikely.

Previous investigations have also described endogenous reactions for the acylation and deacylation of membrane phospholipids (Newkirk & Waite, 1971; Holub & Kuksis, 1978) in a number of plasma membranes, including rat brush-border membranes (O'Doherty, 1979). Although earlier studies had suggested that Ca²⁺ might influence fluidity of hepatocyte plasma membranes via deacylation-reacylation cycles (Livingstone & Schachter, 1980), no direct proof for this assertion was shown. Further studies along these lines in rat small-intestinal brush-border membranes will therefore be necessary to clarify this issue.

Regardless of the mechanism(s) involved, however, in this 'irreversible effect' of Ca²⁺ on fluidity, the present studies demonstrate that this bivalent cation can modulate the fluidity and lipid composition of specific regions of the cell surface of rat enterocyte, i.e. brush-border but not basolateral membranes, via this mechanism. Storch *et al.* (1983) showed that Ca²⁺ could influence the fluidity of contraluminal (sinusoidal and contiguous), but not luminal (canalicular), plasma membranes of rat hepatocytes. It therefore appears that, depending on the cell type, the effects of Ca²⁺ on fluidity by this mechanism may be selective for certain antipodal plasma membranes.

The alterations in plasma-membrane fluidity induced by Ca²⁺ in the present studies were relatively small. Previous observations by our laboratory, however, have demonstrated that similar quantitative changes in fluidity

influenced a number of enzymic activities and transport processes in these membranes (Brasitus *et al.*, 1979; Brasitus & Schachter, 1980a,b, 1982; Brasitus *et al.*, 1986). For example, decreases in *r* values of DPH in the range of 5–15% have been correlated with increases in the specific activities of adenylate cyclase (200%) and guanylate cyclase (400%), as well as increases in the transport of Na⁺-dependent D-glucose (60%) and Na⁺/H⁺ exchange (60%) in those previous studies. It therefore seems reasonable to suggest that these fluidity changes induced by Ca²⁺ may be physiologically important. Further studies of these membranes should clarify questions concerning possible functions and mechanisms for these Ca²⁺-induced alterations in membrane lipid fluidity.

We thank Kimberli Coleman for her excellent technical assistance. We are also grateful to Dolores Gordon and Donna Ellzey for their excellent secretarial support. This investigation was supported by U.S. Public Health Service no. Ca 36745 awarded by the National Cancer Institute, D.H.H.S.

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Received 24 March 1986/2 June 1986; accepted 8 July 1986