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Dexamethasone-induced alterations in lipid composition and fluidity of rat proximal-small-intestinal brush-border membranes

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A series of experiments were conducted to examine the possible effects of subcutaneous administration of the synthetic glucocorticoid dexamethasone (100 μg/day per 100 g body wt.) on the lipid fluidity and lipid composition of rat proximal-small-intestinal brush-border membranes. After 4 days of treatment, membranes and their liposomes prepared from treated animals possessed a greater fluidity than did their control (diluent, 0.9% NaCl) counterparts, as assessed by steady-state fluorescence-polarization techniques using several different fluorophores. Examination of the effects of temperature on the anisotropy values of 1,6-diphenylhexa-1,3,5-triene, using Arrhenius plots, moreover, revealed that the mean break-point temperatures of the treated preparations were approx. 3–4 °C lower than those of their control-preparation counterparts. Changes in the sphingomyelin/phosphatidylcholine (PC) molar ratio as well as in certain of the fatty acids of the PC fraction of treated membranes, secondary to alterations in membrane PC levels and in lysophosphatidylcholine acyltransferase activities respectively, were also noted after dexamethasone administration. These compositional alterations appeared to be responsible, at least in part, for the differences in fluidity noted between treated and control plasma membranes. These results therefore demonstrate that dexamethasone administration can modulate the lipid fluidity and lipid composition of rat proximal-small-intestinal brush-border membranes.

INTRODUCTION

Over the past several years, the importance of lipid–protein interactions in influencing protein-mediated activities of biological membranes, including the rat small- and large-intestinal plasma antipodal membranes, have received increased recognition (Brasitus & Dudeja, 1988). Considerable evidence now exists that alterations in the physical state of the lipid of these membranes can influence a number of important cell-surface functions, including the activities of certain enzymes (Brasitus *et al.*, 1979; Brasitus & Schachter, 1980*a*,*b*), $Na^+ \rightleftharpoons H^+$ exchange and water-permeability (Brasitus *et al.*, 1986) and D-glucose transport (Brasitus *et al.*, 1979; Brasitus & Schachter, 1982). In this regard, recent studies have suggested that glucocorticoids can influence the lipid composition and physical state of membrane lipids of several non-intestinal cell types (Johnston & Melnykovych, 1980; Boullier *et al.*, 1982; Kapilulnik *et al.*, 1986).

Previous studies in a number of laboratories have also demonstrated that corticosteroids can significantly alter the structure and function of the normal digestive system (Charney *et al.*, 1975; Batt & Peters, 1976; Ananna *et al.*, 1979; Scott *et al.*, 1981). In the adult rat intestinal tract, for example, corticosteroids have been shown to affect fluid and electrolyte movement (Charney *et al.*, 1975; Binder, 1978; Field, 1978), increase the activities of certain plasma-membrane-bound enzymes, as well as increase the absorption of hexoses (Charney *et al.*, 1975; Binder, 1978; Field, 1978).

Antenatal (Pang *et al.*, 1985; Neu *et al.*, 1986) or postnatal (Neu *et al.*, 1986) administration of corticosteroids have also recently been shown to decrease the 'lipid fluidity' of rat small-intestinal brush-border membranes. [Note: the term 'lipid fluidity' as applied to anisotropic bilayer membranes is used to denote the relative motional freedom of the lipid molecule or substituents thereof. A more detailed description is given by Schachter (1984). Briefly, as evaluated by steady-state fluorescence polarization of lipid fluorophores, fluidity is assessed via the parameters of the modified Perrin relationship described in the Experimental section. An increase in fluidity corresponds to either a decrease in the correlation time, T_c, or the hindered anisotropy, r_∞, of the fluorophore. Therefore, the term combines the concepts of the 'dynamic and static (lipid order) components' of fluidity.] To date, similar studies have not been conducted in adult rats. The present experiments were therefore performed to determine if administration of dexamethasone influenced the lipid fluidity of adult rat proximal-small-intestinal brush-border membranes as well as to elucidate the mechanism(s) involved in such a corticosteroid-mediated effect.

The results described below demonstrate: (1) that dexamethasone administration was found to increase significantly the lipid fluidity of adult rat proximal-small-intestinal brush-border membranes compared with their control counterparts, as assessed by steady-state fluorescence polarization using several different fluorophores; (2) these changes could, at least partially, be explained by alterations in the sphingomyelin/PC molar ratio, as well

Abbreviations used: PC, phosphatidylcholine ('lecithin'); DPH, 1,6-diphenylhexa-1,3,5-triene; 2-AS, DL-2-(9-anthroyl)stearic acid; 12-AS, DL-12-(9-anthroyl)stearic acid.

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as in the levels of stearic acid, linoleic acid and arachidonic acid in the PC fraction of phospholipids of dexamethasone-treated membranes; and (3) in turn, concomitant increases in PC levels as well as increases in the activities of lysophosphatidylcholine acyltransferase(s) respectively appeared to underlie these membrane-compositional alterations. These results, as well as a discussion of their possible physiological significance, serve as the basis for the present paper.

EXPERIMENTAL

Materials

Dexamethasone was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fatty acids, 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. (Fairlawn, NJ, U.S.A.) or Sigma, unless otherwise indicated.

Membrane preparations

Male albino rats of the Sprague–Dawley strain, weighing 250–300 g, were used for all studies. Animals were injected subcutaneously with either dexamethasone (100 μg/day per 100 g body wt.) or vehicle (0.9% saline) for 4 days. The last injection was administered 2 h before the animals were killed. The animals were starved for 18 h, with water *ad libitum*, killed rapidly by cervical dislocation, and their proximal small intestine excised. Brush-border membranes were then prepared from the intestinal mucosal scrapings, using 10 mM-$MgCl_2$ instead of $CaCl_2$ as the precipitating agent (Brasitus & Dudeja, 1986), as previously described (Brasitus *et al.*, 1979). Purity and comparability of the membrane preparations were assessed by using appropriate marker enzymes (Brasitus *et al.*, 1979). All brush-border membranes were purified 15–20-fold with respect to sucrase and *p*-nitrophenyl phosphatase (alkaline phosphatase) activities compared with the original homogenates. The corresponding specific-activity ratios for succinic dehydrogenase, NADPH:cytochrome *c* reductase and ($Na^+ + K^+$)-dependent ATPase, marker enzymes for mitochondrial, microsomal and basolateral membranes respectively, ranged from 0.40 to 1.30 in all membrane preparations, as previously described (Brasitus *et al.*, 1979). 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 lipids 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 N_2 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 was determined by the procedure of Zlatkis *et al.* (1953). Derivatives of fatty acids of the total lipid extract were prepared by the method of 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 were used to identify retention times (Gartner & Vahouny, 1972).

Fluorescence-polarization studies

Three fluorophores were used: DPH, 2-AS and 12-AS. All compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.) or Molecular Probes Inc. (Junction City, OR, U.S.A.). Steady-state fluorescence-polarization 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 were described previously (Brasitus & Schachter, 1982; Brasitus & Dudeja, 1985*b*). The content of the fluorophores in the preparations was measured fluorimetrically as described by Cogan & Schachter (1981). Final probe/lipid molar ratios ranged from 0.002 to 0.003, and the anisotropy differences noted in the present studies could not be ascribed to differences in probe concentrations in the membranes. Corrections for light-scattering (suspensions minus probe) and the fluorescence in the ambient medium (determined by pelleting the preparations after each measurement) were made routinely, and the combined corrections were less than 3 and 5% respectively of the total fluorescence intensity observed for DPH and the anthroxy-loaded preparations.

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

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

where *r* is the fluorescence anisotropy, r_o is the maximal limiting anisotropy, taken as 0.365 for DPH (Shinitzky & Barenholz, 1974), and 0.285 for the anthroxy probes (2- and 12-AS) (Schachter & Shinitzky, 1977), 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:

$$S = (r_\infty/r_o)^{\frac{1}{2}}$$

as described previously (Van Blitterswijk *et al.*, 1981). The lifetime of the excited-state T_f for each probe in each preparation was estimated by phase fluorimetry at 18 and 30 mH (Spencer & Weber, 1969; Lakowicz *et al.*, 1979) in an SLM 4800 subnanosecond polarization spectrophotometer (SLM-Aminco, Urbana, IL, U.S.A.), as previously described (Storch & Schachter, 1984; Brasitus & Dudeja, 1985*a*). The anisotropy changes noted in these experiments could not be ascribed to differences in lifetimes of these probes. Arrhenius plots of log *r* against $1/T$ were constructed to detect lipid thermotropic temperatures as previously described (Schachter & Shinitzky, 1977; Brasitus *et al.*, 1979).

Enzymic activities

Phospholipase A_2 activity was determined in cellular homogenates by a modification of the method of Tagesson & Sjodahl (1985). Portions (50 μl) of a sonicated dispersion of 6 mM-1-palmitoyl-2-[1-^{14}C]oleoyl PC in water were added to 50 μl of reaction mixture containing 9.0 μmol of Tris/HCl buffer, pH 7.5, 0.2 μmol of $CaCl_2$ and 40–50 μg of protein from cellular homogenates. Reaction mixtures were incubated at 37 °C and the reaction stopped at 90 min by the addition of 2 ml of chloroform/methanol (1:2, v/v). Lipids were extracted by the method of Bligh & Dyer (1959), dried under an N_2 stream and transferred to silica-gel G t.l.c. plates in 50 μl of chloroform. Lipids were then separated by using a mobile phase containing chloroform/methanol/acetic acid/formic acid/water (35:15:6:2:1, by vol.). The uppermost band at the solvent front, corresponding to released radiolabelled oleic acid, was then scraped off, transferred to scintillation vials containing 10 ml of Scintiverse E and counted for radioactivity. Enzyme activity was expressed as nmol of oleic acid produced by hydrolysis of PC/min per mg of protein.

Lysophosphatidylcholine acyltransferase was assayed in cellular homogenates by determining the incorporation of radiolabelled arachidonyl- or linoleoyl-CoA into 1-palmitoyl-*sn*-glycero-3-phosphocholine (palmitoyl PC) as described by de Vries *et al.* (1985). The 50 μl reaction mixtures contained 0.2 mM-1-palmitoyl-*sn*-glycero-3-phosphocholine (taken from a 1 mM sonicated dispersion in water), 10 mM-$MgCl_2$, 65 mM-Tris/HCl, pH 7.4, and 44 μM-[1-^{14}C]arachidonyl-CoA (45.3 mCi/nmol) or 44 μM-[1-^{14}C]linoleoyl-CoA (41.4 mCi/nmol). Reactions were initiated by the addition of 20 μg of homogenate protein and incubated at 37 °C for 20 min in a shaking water bath. Reactions were stopped by addition of 2 ml of chloroform/methanol (1:1, v/v). Lipids were then extracted and separated on t.l.c. plates as described above. Radioactivity in the band corresponding to PC was determined, and enzyme activity was expressed as nmol of PC formed/min per mg of protein. All reactions were linear with respect to time and protein concentrations.

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 dexamethasone administration on brush-border-membrane fluidity

As shown in Table 1, the lipid fluidity of proximal-small-intestinal brush-border membranes of rats treated with dexamethasone was significantly higher than that of control membranes, as assessed by steady-state fluorescence-polarization techniques at 25 °C, using all three fluorophores. As previously discussed (Brasitus & Dudeja, 1985*a*), these probes differ in a number of respects. In this regard, it should be noted that the structural organization of the lipid bilayer of biological membranes appears to hinder the rotation of DPH. Therefore r_∞ values for this fluorophore are high and largely determine *r* (Van Blitterswijk *et al.*, 1981). In contrast, other probes, such as the anthroxy derivatives (2- and 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 (Schachter *et al.*, 1982; Vincent *et al.*, 1982). In the present studies both the 'static' and 'dynamic' components of membrane fluidity, as assessed by r_∞ and *S* values of DPH and *r* values of 2-AS and 12-AS respectively were found to be increased in membranes prepared from treated animals. Differences were also noted in liposomes prepared from the lipid extracts of treated membranes when compared with liposomes prepared from control membranes using DPH (Table 1).

The effects of temperature on the DPH fluorescence anisotropy, *r*, in brush-border membranes and liposomes prepared from the proximal small intestine of treated and control animals are illustrated by representative Arrhenius plots in Fig. 1. At each temperature tested, the mean values for both the treated membranes and their liposomes were significantly lower than their control

Table 1. Fluorescence polarization studies of proximal-small-intestinal brush-border membranes and their liposomes prepared from dexamethasone-treated and control rats

Values are means ± S.E.M. for the numbers (*n*) of preparations examined at 25 °C. Statistical significance: [b] $P < 0.05$ or less compared with corresponding control-intact-membrane value; [c] $P < 0.05$ or less compared with corresponding control-liposome value; [d] $P < 0.05$ or less compared with corresponding treated-intact-membrane value.

Probe	Preparation	(*n*)	Anisotropy (*r*)	Limiting hindered anisotropy (r_∞)	Order parameter (*S*)
DPH	Control intact membranes	(11)	0.260 ± 0.004	0.247 ± 0.005	0.823 ± 0.007
	Treated intact membranes	(10)	0.239 ± 0.006[b]	0.219 ± 0.006[b]	0.775 ± 0.008[b]
	Control liposomes	(5)	0.237 ± 0.010[b]	0.216 ± 0.010[b]	0.769 ± 0.012[b]
	Treated liposomes	(5)	0.212 ± 0.009[c,d]	0.183 ± 0.009[c,d]	0.708 ± 0.011[c,d]
2-AS	Control intact membranes	(10)	0.232 ± 0.007	–	–
	Treated intact membranes	(9)	0.212 ± 0.007[b]	–	–
	Control liposomes	(5)	0.191 ± 0.008[b]	–	–
	Treated liposomes	(6)	0.164 ± 0.010[c,d]	–	–
12-AS	Control intact membranes	(10)	0.147 ± 0.002	–	–
	Treated intact membranes	(9)	0.137 ± 0.003[b]	–	–
	Control liposomes	(6)	0.125 ± 0.005[b]	–	–
	Treated liposomes	(5)	0.110 ± 0.006[c,d]	–	–

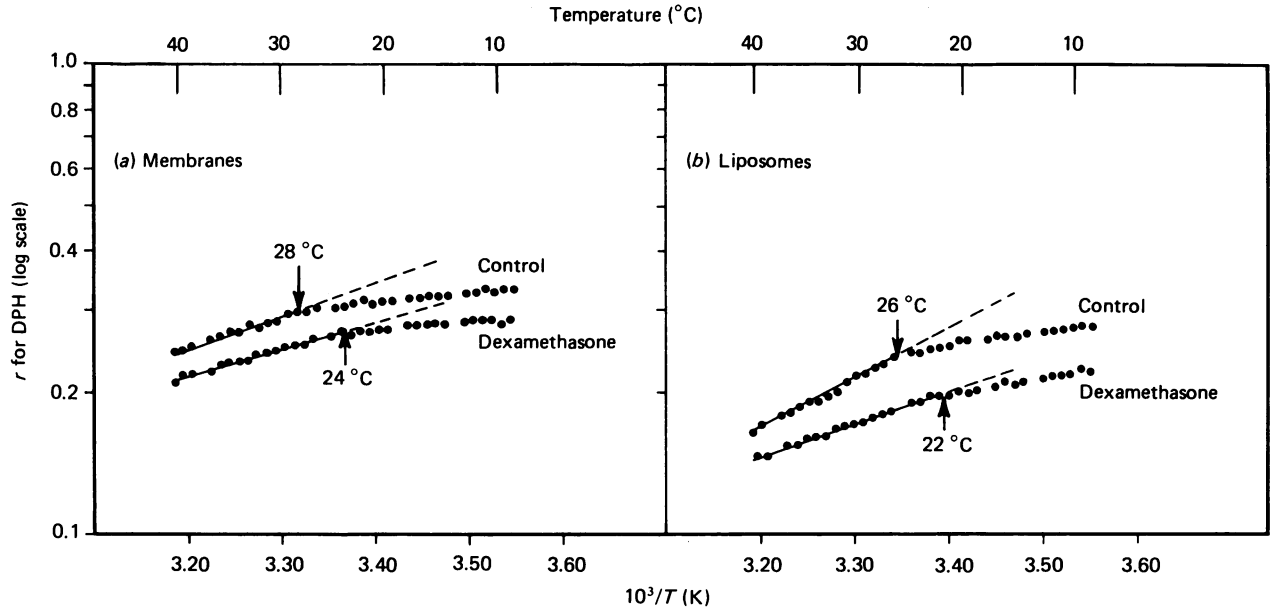

Fig. 1. Representative Arrhenius plots of the anisotropy (*r*) values of DPH (*a*) for isolated brush-border membranes prepared from the proximal small intestine of control (upper curve) and dexamethasone-treated (lower curve) animals and (*b*) for control (upper curve) and treated (lower curve) liposomes prepared from lipid extracts of these membranes

Table 2. The effects of temperature on the DPH fluorescence-anisotropy values in brush-border membranes and liposomes prepared from the proximal small intestine of dexamethasone-treated and control rats

Values represent means ± S.E.M. for the numbers of preparations (*n*) given. **P* < 0.05 compared with corresponding control values.

Preparation	(*n*)	Break-point temperature (°C)
Intact membrane		
Control	(4)	28.0 ± 1.0
Treated	(4)	24.5 ± 0.8*
Liposomes		
Control	(3)	26.3 ± 0.3
Treated	(3)	22.0 ± 0.6*

Table 3. Compositional parameters of control and dexamethasone-treated proximal-small-intestinal brush-border membranes

Values represent means ± S.E.M. for the numbers of preparations (*n*) given. The saturation index was calculated as $a/(bc)$, where a is the total number of saturated residues, b is the sum of the number of each type of unsaturated residue and c is the number of double bonds in that residue. **P* < 0.05 or less compared with control values.

Parameter	(*n*)	Control membranes	Treated membranes
Molar ratio			
Cholesterol/phospholipid	(6)	0.82 ± 0.05	0.80 ± 0.05
Sphingomyelin/PC	(6)	0.50 ± 0.04	0.39 ± 0.05*
Saturation index	(6)	0.40 ± 0.03	0.32 ± 0.01*
Protein/lipid (w/w) ratio	(4)	1.00 ± 0.11	1.12 ± 0.12

counterparts. A change in the slope of the Arrhenius plot, indicative of a lipid thermotropic transition (Schachter & Shinitzky, 1977; Brasitus & Schachter 1980*a*) was observed in all preparations examined. The mean break-point temperatures of the treated preparations were approx. 3–4 °C lower than their control counterparts, as indicated in Table 2. This decrease in thermotropic transition temperatures in the treated preparations would be in accord with their greater lipid fluidity (Brasitus *et al.*, 1984).

Membrane-composition studies

In the present experiments, proximal-small-intestinal brush-border membranes of rats administered dexamethasone were found to possess a higher fluidity than their control counterparts. In this regard, previous studies in model and biological membranes have correlated a high lipid fluidity with low cholesterol/phospholipid and sphingomyelin/PC molar ratios, with less-saturated or shorter acyl chains in phospholipids (Hubbell & McConnell, 1971; Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978), as well as with low protein/lipid (w/w) ratios (Brasitus *et al.*, 1980). We therefore decided to examine and compare these parameters in treated and control membranes. As shown in Table 3, control and treated membranes showed similar cholesterol/phospholipid molar ratios and protein/lipid (w/w) ratios. Treated membranes, however, were found to possess a lower sphingomyelin/PC molar ratio than that of control membranes. The latter appeared to be secondary to an increase in PC levels in treated membranes (Table 4). Moreover, treated membranes were also found to have a

Table 4. Analysis of relative percentages of individual neutral lipids and phospholipids extracted from dexamethasone-treated and control brush-border membranes

Results are means ± S.E.M. for six individual preparations of each membrane. * $P < 0.05$ compared with control values.

Lipid	Percentage (w/w) of total lipid	
	Control membranes	Treated membranes
Cholesterol	26.74±1.14	26.81±1.40
Cholesterol esters	1.25±0.12	1.23±0.09
Triacylglycerol	1.99±0.34	1.78±0.31
Non-esterified fatty acids	2.94±0.41	2.82±0.38
Phosphatidylethanolamine	13.57±0.76	12.96±0.69
Phosphatidylinositol	5.44±0.99	5.73±0.97
Phosphatidylserine	12.49±0.91	11.85±1.21
PC	22.54±1.01	26.34±0.99*
Sphingomyelin	11.31±0.82	10.40±0.89

lower saturation index than control membranes (Table 3). Analysis of the total fatty acids of these membranes revealed that treated preparations possessed significantly higher percentages of linoleic acid ($C_{18:2}$) and arachidonic acid ($C_{20:4}$) and a lower percentage of stearic acid ($C_{18:0}$) than did control membranes (results not shown). These differences in fatty acids appeared to be responsible for the lower saturation index seen in dexamethasone-treated membranes, and, along with the lower sphingomyelin/PC molar ratio, appeared to explain, at least in part, the differences in fluidity present between control and treated membranes (Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978).

To explore further the fatty acid differences noted between these membranes, the individual fatty acids of PC and phosphatidylethanolamine were examined and compared (Table 5). As shown in Table 5, no significant differences were noted in the individual fatty acids of the phosphatidylethanolamine fraction of these membranes. In contrast with these findings, however, the relative percentages of linoleic acid and arachidonic acid were found to be significantly increased, and stearic acid decreased, in the PC fraction of treated membranes compared with their control counterparts (Table 5).

Effect of dexamethasone on enzymic activities

Previous studies in other tissues have suggested that corticosteroids might influence the enzymic activities involved in the fatty acyl deacylation–reacylation cycle of PC (Rooney *et al.*, 1976; Hirata *et al.*, 1980). In view of dexamethasone-induced alterations noted in the fatty acids of this fraction of brush-border membranes (Table 5), we decided to examine and compare these enzymic activities in intestinal homogenates of treated and control

Table 5. Analysis of individual fatty acids of phosphatidylethanolamine and PC fractions of phospholipids prepared from extracts of control and dexamethasone-treated proximal-small-intestinal brush-border membranes

Values represent means ± S.E.M. for four separate preparations of each membrane type. * $P < 0.05$ or less compared with control values.

Fatty acid	Composition (%, w/w, of total lipid)			
Fraction ...	Phosphatidylethanolamine		PC	
Membranes ...	Control	Treated	Control	Treated
$C_{16:0}$	19.1±0.5	18.9±1.1	35.7±1.2	36.1±0.9
$C_{18:0}$	44.8±1.6	45.5±1.6	37.0±0.3	30.3±0.8
$C_{18:1}$	13.2±0.2	12.9±0.4	9.5±0.6	8.3±0.5
$C_{18:2}$	10.6±1.1	11.0±0.3	11.7±0.2	16.1±0.2*
$C_{20:4}$	12.2±0.8	11.8±1.2	6.1±0.6	9.2±0.3*

Table 6. Specific activities of phospholipase A_2 and lysophosphatidylcholine acyltransferases in proximal-small-intestinal mucosal homogenates of control and dexamethasone-treated rats

Values represent means ± S.E.M. for the numbers of preparations (n) of each membrane. * $P < 0.05$ or less compared with control values.

Enzymic activity	(n)	Membranes ...	Specific activity (nmol/min per mg of protein)	
			Control	Treated
Phospholipase A_2	(10)		1.42±0.09	1.20±0.06
Linoleoyl-CoA:lysophosphatidyl choline acyltransferase	(6)		1.87±0.27	2.31±0.18*
Arachidonyl-CoA:lysophosphatidyl choline acyltransferase	(13)		0.64±0.09	1.15±0.14*

rats. As shown in Table 6, phospholipase A_2 activity was not significantly altered by dexamethasone administration. The specific activity of lysophosphatidylcholine acyltransferase(s), with linoleoyl-CoA and arachidonyl-CoA as acyl donors, however, was significantly increased by dexamethasone treatment. These data therefore suggest that alterations in the activity of the latter enzyme(s) may, at least in part, be responsible for the dexamethasone-induced fatty acid alterations seen in the PC fraction of rat proximal-small-intestinal brush-border membranes.

DISCUSSION

Previous studies in our laboratory (Brasitus *et al.*, 1984) and by others (Pang *et al.*, 1985; Schwarz *et al.*, 1982, 1984, 1985) have demonstrated a decrease in small-intestinal luminal-plasma-membrane fluidity, as assessed by both e.s.r. and steady-state fluorescence-polarization techniques, during postnatal development in rats and rabbits. Thus the luminal membranes of younger animals possessed a greater lipid fluidity than their adult counterparts. In this regard, fairly recent studies by Pang *et al.* (1985) and Neu *et al.* (1986) have shown that antenatal and/or postnatal administration of various glucocorticoids led to a decrese in the lipid fluidity of small-intestinal brush-border membranes of rabbits and rats respectively. On the basis of these latter observations, those authors (Pang *et al.*, 1985; Neu *et al.*, 1986) suggested that exposure to glucocorticoids *in utero* or in the postnatal period accelerated the maturation of the physical state of luminal lipids of enterocytes. In contrast with these findings, Kapilulnik *et al.* (1986) found that dexamethasone, administered to pregnant rats during the last week of pregnancy, markedly increased the fluidity of liver microsomal membranes in the postnatal period. Taken together, these results suggest that exposure to glucocorticoids can modulate the fluidity of plasma and intracellular membranes in young animals, albeit in opposite directions. Similar studies on the effects of these agents on the fluidity of intestinal plasma membranes prepared from adult animals had, until now, not been performed.

In the present experiments, brush-border membranes and their liposomes, prepared from the proximal small intestine of dexamethasone-treated rats, displayed a greater lipid fluidity than their control adult counterparts. Both the 'static' and 'dynamic' components of fluidity in treated preparations were increased, as assessed by r_∞ and S values of DPH and r values of 2-AS and 12-AS respectively. Furthermore, greater fluidity for the treated preparation was also demonstrated by finding a lowering of their thermotropic transition temperatures (Fig. 1). As previously discussed (Brasitus *et al.*, 1980), steady-state fluorescence polarization, the technique used in the present studies, detects only the lower critical temperature of broad transitions observed by differential scanning calorimetry in rat enterocyte plasma membranes. The transition temperatures detected in treated and control preparations therefore probably represent the lower critical temperatures of the lipid transitions.

The latter findings, together with the data of Neu *et al.* (1986) and Pang *et al.* (1985), would strongly suggest that age appears to be an important variable in the fluidity alterations seen after corticosteroid administration in small-intestinal brush-border membranes. In this regard, previous studies (Herbst & Koldovsky, 1972; Henning & Kretschmer, 1973) have also suggested that the timing of administration of corticosteroids may be critical to their effect on rat small-intestinal brush-border-membrane enzymic activities.

Unfortunately, neither Pang *et al.* (1985) nor Neu *et al.* (1986) examined the lipid compositional parameters likely to be responsible for the corticosteroid-induced alterations in fluidity seen in their small-intestinal membranes prepared from young animals. Kapilulnik *et al.* (1986), however, found that dexamethasone increased the lipid fluidity of rat hepatic microsomal membranes, at least in part, by increasing the levels of polyunsaturated fatty acids in these membranes. Moreover, the latter authors suggested that these fatty acid changes might be mediated via alterations in the activities of enzymes involved in fatty acid desaturation and/or reacylation–deacylation, although these activities were not directly measured.

In the present studies, brush-border membranes of dexamethasone-treated rats were also characterized by alterations in the composition of certain fatty acids in the PC fraction of their membrane phospholipids. Furthermore, in agreement with results of previous studies performed on the effects of corticosteroids on rabbit lung tissue (Rooney *et al.*, 1976), concomitant with these lipid-compositional changes, the specific activities of lysophosphatidylcholine acyltransferase(s), with linoleoyl-CoA and arachidonic-CoA as acyl donors, were found to be increased by dexamethasone administration. It would therefore appear reasonable to suggest that dexamethasone-induced increases in these enzymic activities may, at least in part, be responsible for the fatty acid changes noted in the PC fraction of treated membranes. In the present experiments, fatty acyl desaturase activities were not studied. In this regard, we have detected fatty acid desaturase activities in the microsomes (microsomal fractions) of rat enterocytes and colonocytes (Ehrlich *et al.*, 1985). Although alterations in these activities might also theoretically be responsible for certain of the fatty acid changes noted in the dexamethasone-treated membranes, given the very low activities of these enzymes present in rat enterocytes (Ehrlich *et al.*, 1985), we believe this possibility is unlikely.

In agreement with results of previous studies performed on rabbit lung (Rooney *et al.*, 1976), dexamethasone was also found to decrease the sphingomyelin/PC molar ratio of proximal-small-intestinal brush-border membranes by increasing the levels of PC in the treated membranes. Previous studies in rabbit (Rooney *et al.*, 1976) and rat fetal lung (Post *et al.*, 1986) demonstrated that glucocorticoid administration stimulated the activity of cholinephosphate cytidylyltransferase, but had no effect on the activities of pulmonary choline kinase and choline-phosphotransferase. Whether similar alterations in cholinephosphate cytidylyltransferase activity are responsible for the increases in PC seen in the brush-border membranes of treated animals in the present study must await further experiments. Regardless of the exact mechanism(s) involved, however, it would appear that dexamethasone treatment produces changes in the level of PC as well as in certain fatty acids of this phospholipid fraction, which, in turn, increase the lipid fluidity of proximal-small-intestinal brush-border membranes.

In this regard it should be noted that, although the

alterations in proximal-small-intestinal brush-border-membrane fluidity induced by dexamethasone in the present studies were relatively small, previous observations in our laboratory have shown that similar quantitative changes in fluidity influenced a number of enzymic and transport processes in these plasma membranes (Brasitus *et al.*, 1979; Brasitus & Schachter, 1980*a*,*b*, 1982). For example, decreases in *r* values of DPH in the range 5–15% have been correlated with increases in the specific activities of guanylate cyclase (400%) as well as increases in the Na^+-dependent transport of D-glucose (60%) in those previous studies. It therefore seems reasonable to suggest that the present fluidity alterations induced by dexamethasone may be physiologically important. Further studies should clarify questions concerning possible functions and mechanism(s) for the corticosteroid-induced alterations in lipid fluidity and lipid composition of this intestinal brush-border membrane.

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REFERENCES

Ames, B. N. & Dubin, D. T. (1960) J. Biol. Chem. **235**, 769–775
Ananna, A., Eloy, R., Bouchet, P., Clendinnen, G. & Crenier, J. F. (1979) Lab. Invest. **41**, 83–88
Batt, R. M. & Peters, T. J. (1976) Clin. Sci. **50**, 511–523
Binder, H. J. (1978) Gastroenterology **75**, 212–217
Bligh, E. G. & Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917
Boullier, J. A., Melnykovych, G. & Barias, B. G. (1982) Biochim. Biophys. Acta **692**, 278–286
Brasitus, T. A. & Dudeja, P. K. (1985*a*) Arch. Biochem. Biophys. **240**, 483–488
Brasitus, T. A. & Dudeja, P. K. (1985*b*) J. Biol. Chem. **260**, 12405–12409
Brasitus, T. A. & Dudeja, P. K. (1986) Biochem. J. **239**, 625–631
Brasitus, T. A. & Dudeja, P. K. (1988) in Advances in Membrane Fluidity (Aloia, R. C., Curtaina, C. C. & Gordon, L. M., eds.), Alan R. Liss, New York, in the press
Brasitus, T. A. & Schachter, D. (1980*a*) Biochemistry **19**, 2763–2769
Brasitus, T. A. & Schachter, D. (1980*b*) Biochim. Biophys. Acta **774**, 138–146
Brasitus, T. A. & Schachter, D. (1982) Biochemistry **21**, 2241–2246
Brasitus, T. A., Schachter, D. & Mamouneas, T. G. (1979) Biochemistry **18**, 4136–4144
Brasitus, T. A., Tall, A. R. & Schachter, D. (1980) Biochemistry **19**, 1256–1261
Brasitus, T. A., Yeh, K. Y., Holt, P. R. & Schachter, D. (1984) Biochim. Biophys. Acta **778**, 341–348
Brasitus, T. A., Dudeja, P. K., Worman, H. J. & Foster, E. S. (1986) Biochim. Biophys. Acta **885**, 16–24
Charney, A. N., Kinsey, M. D., Meyers, L., Gianella, R. A. & Gots, R. E. (1975) J. Clin. Invest. **56**, 6353–6660
Cogan, U. & Schachter, D. (1981) Biochemistry **20**, 6396–6406
de Vries, A. C. J., Batenburg, J. J. & Van Golde, L. M. G. (1985) Biochim. Biophys. Acta **833**, 93–99
Ehrlich, J. B., Dahiya, R. & Brasitus, T. A. (1985) Gastroenterology **88**, 1373
Field, M. (1978) Gastroenterology **75**, 317–319
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) J. Biol. Chem. **226**, 497–509
Gartner, S. L. & Vahouny, G. (1972) Am. J. Physiol. **222**, 1121–1124
Henning, S. J. & Kretschmer, N. (1973) Enzyme **15**, 3–23
Herbst, J. J. & Koldovsky, O. (1972) Biochem. J. **126**, 471–476
Heyn, M. P. (1979) FEBS Lett. **108**, 359–364
Hirata, F., Schiffman, E., Venkatasubramanian, K., Salomon, D. & Axelrod, J. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 2533–2536
Hubbell, W. L. & McConnell, H. M. (1971) J. Am. Chem. Soc. **93**, 314–326
Jahnig, F. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 6361–6365
Johnston, D. & Melnykovych, G. (1980) Biochim. Biophys. Acta **596**, 320–324
Kapilulnik, J., Weil, E. & Rabinowitz, R. (1986) Biochem. J. **239**, 41–45
Katz, S. S., Shippley, G. G. & Small, D. M. (1976) J. Clin. Invest. **58**, 200–211
Lakowicz, J. R., Prendergast, F. G. & Hogen, D. (1979) Biochemistry **18**, 508–519
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Neu, J., Ozaki, C. K. & Angelides, K. J. (1986) Pediatr. Res. **20**, 79–82
Pang, K. Y., Newman, A. P., Udall, J. N. & Walker, W. A. (1985) Am. J. Physiol. **249**, G85–G91
Post, M., Barsoumanian, A. & Smith, B. T. (1986) J. Biol. Chem. **261**, 2179–2184
Rooney, S. A., Gobran, L., Gross, I., Wai-Lee, T. S., Nardone, L. L. & Motoyama, E. K. (1976) Biochim. Biophys. Acta **450**, 121–130
Schachter, D. (1984) Hepatology **4**, 140–151
Schachter, D. & Shinitzky, M. (1977) J. Clin. Invest. **59**, 536–548
Schachter, D., Cogan, U. & Abbot, R. E. (1982) Biochemistry **21**, 2146–2150
Schwarz, S. M., Ling, S., Hostetler, B., Lee, L. & Watkins, J. B. (1982) Gastroenterology **82**, 1174
Schwarz, S. M., Ling, S., Hostetler, B., Draper, J. P. & Watkins, J. B. (1984) Gastroenterology **86**, 1544–1551
Schwarz, S. M., Hostetler, B., Ling, S., Moore, M. & Watkins, J. B. (1985) Am. J. Physiol. **248**, G200–G207
Scott, J., Batt, R. M., Maddison, Y. E. & Peter, T. J. (1981) Am. J. Physiol. **241**, G306–G312
Shinitzky, M. & Barenholz, Y. (1974) J. Biol. Chem. **249**, 2652–2657
Shinitzky, M. & Barenholz, Y. (1978) Biochim. Biophys. Acta **515**, 367–394
Shinitzky, M. & Inbar, M. (1976) Biochim. Biophys. Acta **433**, 133–149
Spencer, R. D. & Weber, G. (1969) Ann. N.Y. Acad. Sci. **158**, 361–376
Storch, J. & Schachter, D. (1984) Biochemistry **23**, 1165–1170
Tagesson, C. & Sjodahl, R. (1985) Scand. J. Gastroenterol. **20**, 25–30
Van Blitterswijk, W. J., Van Hoeven, R. P. & Van Der Meer, B. W. (1981) Biochim. Biophys. Acta **644**, 323–332
Vincent, M., DeForesta, B., Gallay, J. & Alfse, A. (1982) Biochem. Biophys. Res. Commun. **107**, 914–921
Zlatkis, A., Zak, B. & Boyle, A. J. (1953) J. Lab. Clin. Med. **41**, 486–492

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