Biochem. J. (1988) 253, 401–408 (Printed in Great Britain)

Dexamethasone influences the lipid fluidity, lipid composition and glycosphingolipid glycosyltransferase activities of rat proximal-small-intestinal Golgi membranes

Pradeep K. DUDEJA, Rajvir DAHIYA, Michael D. BROWN and Thomas A. BRASITUS*
Department of Medicine, University of Chicago Hospitals and Clinics, and Michael Reese Hospital and Medical Center, Chicago, IL 60637, U.S.A.

Experiments were performed to examine the effects of subcutaneous administration of the synthetic glucocorticoid dexamethasone (100 μg/day per 100 g body wt.) on the lipid fluidity, lipid composition and glycosphingolipid glycosyltransferase activities of rat proximal-small-intestinal Golgi membranes. After 4 days of treatment, Golgi membranes and liposomes prepared from treated rats were found to possess a greater fluidity than their control (diluent or 0.9% NaCl) counterpart, as assessed by steady-state fluorescence-polarization techniques using three different fluorophores. Moreover, analysis of the effects of temperature on the anisotropy values of 1,6-diphenylhexa-1,3,5-triene, using Arrhenius plots, demonstrated that the mean break-point temperatures of treated preparations were 4–5 °C lower than those of control preparations. Changes in the fatty acyl saturation index and double-bond index of treated membranes, secondary to alterations in stearic acid, linoleic acid and arachidonic acid, at least in part, appeared to be responsible for the differences in fluidity noted between treated and control Golgi membranes. Concomitant with these fluidity and lipid-compositional alterations, treated membranes possessed higher specific activities of UDP-galactosyl-lactosylceramide galactosyltransferase and CMP-*N*-acetylneuraminic acid:lactosylceramide sialyltransferase than their control counterparts. Experiments utilizing benzyl alcohol, a known fluidizer, furthermore suggested that the fluidity alteration induced by dexamethasone may be responsible for the increased activity of the former, but not the latter, glycosphingolipid glycosyltransferase.

INTRODUCTION

Glucocorticoid hormones have been shown to influence the structure and/or function of a wide variety of cell types, including small- and large-intestinal epithelial cells (Charney *et al.*, 1975; Ananna *et al.*, 1979; Scott *et al.*, 1981; Lentze *et al.*, 1985; Dudeja *et al.*, 1987). For example, these hormones have been shown to affect intestinal fluid, electrolyte and nutrient absorption (Charney *et al.*, 1975; Binder, 1978; Field, 1978; Meneely & Ghishan, 1982; Dudeja *et al.*, 1987), epithelial-cell maturation (Henning & Sims, 1979) and the activities of certain plasma-membrane-bound enzymes (Lebenthel *et al.*, 1972; Daniels *et al.*, 1973; Marnane *et al.*, 1981). Recently, our laboratory has also demonstrated that administration of the synthetic glucocorticoid dexamethasone altered the glycosphingolipid composition of the rat proximal-small-intestinal mucosa (Dahiya & Brasitus, 1987) as well as changing the lipid fluidity and phospholipid composition of rat small- and large-intestinal brush-border membranes (Brasitus *et al.*, 1987*b*; Dudeja *et al.*, 1987). [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 either to 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.]

The Golgi apparatus is an important intracellular organelle which has been shown to be involved in a number of cellular processes, including phospholipid biosynthesis (Higgins & Fieldsend, 1987), lipoprotein assembly and transcellular transport (Higgins & Hutson, 1984), calcium binding and translocation (Freedman *et al.*, 1977), lysosome formation (Farquhar & Palade, 1981), as well as glycosylation of proteins and lipids (Fleischer & Fleischer, 1970; Burczak *et al.*, 1984). The enzymic activities responsible for the glycosylation of proteins, namely glycoprotein glycosyltransferase, and lipids, namely glycosphingolipid glycosyltransferases, in the Golgi apparatus appear to be membrane-bound (Burzak *et al.*, 1984). Earlier studies have suggested that alterations in the physical state of rat hepatic Golgi membranes could be correlated with changes in the activities of certain glycoprotein glycosyltransferases (Mitranic *et al.*, 1982). Although previous studies have shown that various membrane-bound enzymes involved in glycosphingolipid synthesis may be modulated by a number of different factors, including acceptor substrate, bivalent cations, availability of sugar nucleotides or phosphorylation–dephosphorylation reactions (Yusefet *et al.*, 1983; Burzak *et al.*, 1984), the possible role of

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

* To whom correspondence and reprint requests should be addressed.

membrane fluidity in the regulation of Golgi membrane glycosphingolipid glycosyltransferase activities has not been explored.

In this regard, our laboratory has recently characterized the lipid composition and lipid fluidity of rat proximal-small-intestinal Golgi membranes (Brasitus *et al.*, 1988). Little is known, however, about the factors which regulate the lipid composition, lipid fluidity and lipid–protein interactions of these membranes. In view of our earlier studies (see above), which demonstrated that dexamethasone could influence the lipid composition and physical state of the lipid of small- and large-intestinal plasma membranes, it was of interest to examine the effect of dexamethasone on these biochemical and biophysical parameters as well as on the activities of glycosphingolipid glycosyltransferases present in rat small-intestinal Golgi membranes.

The results of these present investigations demonstrate that Golgi membranes prepared from dexamethasone-treated animals have a higher lipid fluidity than their control counterparts, as assessed by steady-state fluorescence-polarization techniques using three different lipid-soluble fluorophores. The differences in fluidity of these membranes appear to be due, at least in part, to a greater degree of unsaturation of the fatty acids in the membranes of treated animals. Furthermore, concomitant with these changes in membrane lipid composition and fluidity, the activities of certain Golgi membrane-bound glycosphingolipid glycosyltransferases were also altered by dexamethasone treatment. These results, as well as a discussion of their possible physiological significance, form the basis of the present paper.

EXPERIMENTAL

Materials

Dexamethasone was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DL-2-(9-Anthroyl)stearic acid (2-AS) and DL-12-(9-anthroyl)stearic acid (12-AS) were purchased from Molecular Probes (Eugene, OR, U.S.A.). 1,6-Diphenylhexa-1,3,5-triene (DPH) was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Fatty acid methyl esters, g.l.c. columns and lipid standards were all purchased from Supelco (Bellefonte, PA, U.S.A.), NuChek Prep (Elysian, MN, U.S.A.) and/or Applied Science Corp. (State College, PA, U.S.A.). All other chemicals were obtained from Sigma Chemical Co. or Fisher Chemical Co. (Firlawn, NJ, U.S.A.), unless otherwise indicated.

Animals and steroid administration

Male Sprague–Dawley rats (250–300 g) were maintained on Purina rat chow diet *ad libitum*. The treated and control groups received subcutaneous injections (100 μg/day per 100 g body wt) of dexamethasone in 0.9% NaCl or diluent respectively for 4 days. The final injection was given to rats 2 h before they were killed on day 4.

Preparation of Golgi membranes

Rats were fasted 18 h with water *ad libitum* before being killed. The proximal halves of the small intestines were removed, and epithelial cells were then obtained by a method which combined mild mechanical dissociation with bivalent-cation chelation (Weiser, 1973). After washing extensively, the epithelial cells were homogenized with a Polytron homogenizer (setting 8, 1 min), and Golgi membranes were prepared by differential centrifugation, followed by sorbitol-density-gradient centrifugation as described by Freedman *et al.* (1977). The purity of the membrane suspensions and the degree of contamination with intracellular organelles were assessed with appropriate marker enzymes. The specific-activity ratio [(purified Golgi membrane)/(crude homogenate)] for the Golgi marker enzymes galactosyltransferase (Freedman *et al.*, 1977), CMP-*N*-acetylneuraminic acid:lactosylceramide sialyltransferase and UDP-galactosyl-lactosylceramide galactosyltransferase (Keenan *et al.*, 1974) were 16–32 in all membrane preparations and did not significantly differ between control and treated preparations. The corresponding values for succinic dehydrogenase, NADPH-cytochrome *c* reductase, acid phosphatase, sucrase and Na^+,K^+-ATPase, marker enzymes for mitochondrial, microsomal, lysosomal, brush-border and basolateral membranes respectively ranged from 0.6–2.0 in all membrane preparations. Membranes were then suspended in appropriate buffers and used immediately. Protein was estimated by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Liposomes

Liposomes of Golgi membranes were prepared as previously described (Brasitus *et al.*, 1979). Suspensions of lipids extracted from Golgi membranes were placed in phosphate-buffered saline, pH 7.4 and sonicated for 10 min under N_2 at 4 °C. Thereafter the preparations were centrifuged at 10000 ***g*** for 10 min, and the resulting supernatant used for fluorescence-polarization studies (see below).

Enzyme assays

Galactosyltransferase activity was measured with ovalbumin as exogenous acceptor by the method of Kim *et al.* (1972) as modified by Podolsky & Weiser (1975). Na^+,K^+-ATPase, NADPH:cytochrome *c* reductase, succinic dehydrogenase, acid phosphatase and sucrase were assayed as previously described (Brasitus *et al.*, 1979; Brasitus & Keresztes, 1984). CMP-*N*-acetylneuraminic acid:lactosylceramide sialyltransferase was assessed by the method of Kaufman & Basu (1966), as previously optimized for rat intestinal tissue (Dahiya *et al.*, 1987), using the following reaction mixture (in μmol, unless otherwise stated): lactosylceramide, 0.1; [^{14}C]CMP-*N*-acetylneuraminic acid, 0.05 (3.6×10^6 c.p.m./μmol); Tween 80/Triton CF-54 (1:2, w/w), 0.6 mg; cacodylate/HCl buffer, pH 6.35, 15; $MgCl_2$, 0.1; and 50–100 μg of enzyme protein, in final volumes of 100 μl. UDP-galactosyl-lactosylceramide galactosyltransferase was assayed as described (Dahiya *et al.*, 1987) with the following reaction mixture (in μmol, unless otherwise specified); Triton CF-54/Tween 80 (2:1 v/v), 0.6 mg; lactosylceramide, 0.05; $MnCl_2$, 1.0; cacodylate/HCl buffer (pH 6.5), 15.0; UDP-[^{3}H]-galactose (5 Ci/mol), 50 nmol and 50–100 μg of membrane protein in a final volume of 100 μl. Reaction mixtures were then incubated at 37 °C for 3 h and reactions were terminated by the addition of 0.1 ml of methanol. Reaction products were then separated by descending paper chromatography and the enzyme's specific-activity values were calculated from the radio-

Table 1. Fluorescence-polarization studies of rat proximal-small-intestinal Golgi membranes and their liposomes after administration of dexamethasone or diluent

Values are means ± S.E.M. for four to six separate membrane preparations or liposomes of each group measured at 25 °C. *$P < 0.05$ or less compared with control values.

Probe	Preparations	Control		Dexamethasone	
		r	r_∞	r	r_∞
DPH	Intact membranes	0.260 ± 0.005	0.247 ± 0.005	0.210 ± 0.004*	0.180 ± 0.003*
	Liposomes	0.235 ± 0.002	0.213 ± 0.002	0.178 ± 0.002*	0.137 ± 0.002*
2-AS	Intact membranes	0.202 ± 0.010	–	0.180 ± 0.009*	–
12-AS	Intact membranes	0.115 ± 0.003	–	0.104 ± 0.003*	–

activity (c.p.m.) in sugars transferred to lipids as previously described (Dahiya *et al.*, 1987). All assay conditions were chosen to assure linearity with respect to time and protein concentration.

Fluorescence-polarization studies

The lipid-soluble fluorophores DPH, 2-AS and 12-AS were used for the present studies. The methods used to load the membranes and liposomes and to quantify the fluorescence anisotropy in Perkin–Elmer 650–40 fluorescence spectrophotometer have been described (Brasitus & Dudeja, 1985; Brasitus *et al.*, 1986). The amount of each fluorophore in the membranes and their liposomes was estimated fluorimetrically as described by Cogan & Schachter (1981). Final fluorophore/lipid molar ratios ranged from 0.001 to 0.003 in all preparations. Corrections for light-scattering and for fluorescence in the ambient medium were also routinely made as previously described (Brasitus *et al.*, 1979). Fluorescence polarization was expressed as the fluorescence anisotropy, r (Brasitus & Schachter, 1980). 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) and 0.285 for 2-AS and 12-AS (Schachter *et al.*, 1982)], r_∞ is the limiting hindered anisotropy, T_c, the correlation time and T_f, the mean lifetime of the excited state. Values of r_∞ for DPH were calculated from r values as previously described by Van Blitterswijk *et al.* (1981). No change in the excited-state lifetimes, as assessed by total fluorescence intensity, was demonstrated for all the three fluorophores in all preparations in the present studies.

The temperature-dependence of the fluorescence anisotropy, r, of DPH was also determined over the range 0–40 °C for Golgi membranes and liposomes of control and dexamethasone-treated rats. The logarithm of anisotropy was then plotted against $1/T$ (K^{-1}) to obtain Arrhenius plots as previously described (Brasitus *et al.*, 1979).

Membrane compositional studies

Total lipids were extracted from the Golgi membranes by the method of Folch *et al.* (1957). Phospholipids (Ames & Dubin, 1960) and cholesterol (Zlatkis *et al.*, 1953) were measured as previously described. The neutral and phospholipid composition of the extracts was examined by quantitative t.l.c. according to the procedure of Katz *et al.* (1976) and Schwarz *et al.* (1986) respectively. To determine the acyl-chain composition, fatty acids of the total lipid extracts were derivatized as described by Gartner & Vahouny (1972). The fatty acid methyl esters were quantified on a Hewlett–Packard 5790A gas–liquid chromatograph equipped with a flame-ionization detector and interfaced with a Hewlett–Packard 3390A integrator as described by Gartner & Vahouny (1972), authentic fatty acid methyl ester standards being used to identify retention times.

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

Fluorescence polarization studies

As evaluated by steady-state fluorescence polarization of lipid fluorophores, 'fluidity' has usually been assessed by the fluorescence anisotropy, r, without resolution of the components which determine r. As discussed previously (Brasitus *et al.*, 1979), within certain limitations r values vary inversely with fluidity. Previous studies with the rod-like fluorophore DPH, however, have shown that the rotations of this probe are hindered in artificial and biological membranes (Lackowicz *et al.*, 1979; Heyn, 1979; Jahnig, 1979). Therefore the fluorescence anistropy of such a fluorophore is not adequately described by the Perrin equation, but by a modified relationship (see the Experimental section). The r_∞ values of DPH in membranes are high and largely determine r (Heyn, 1979; Jahnig, 1979; Van Blitterswijk *et al.*, 1981) and therefore can be used to assess the 'static' component of fluidity (Schachter, 1984). Unlike DPH, the anthroyloxy probes (2-AS and 12-AS) yield relatively low values of r_∞ in bilayer membranes (Schachter *et al.*, 1982) and can therefore be used to assess the 'dynamic' component of fluidity (Schachter, 1984).

As shown in Table 1, both the 'static' and 'dynamic' components of fluidity were significantly greater in Golgi membranes prepared from dexamethasone-treated rats compared with their control counterparts, as determined

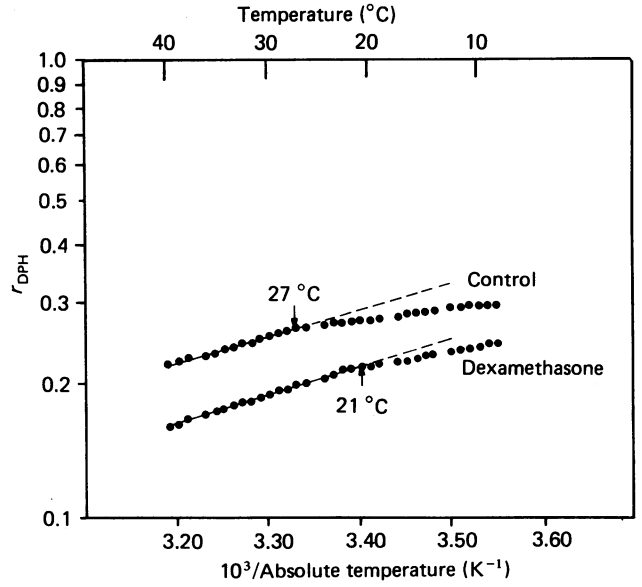

Fig. 1. Representative Arrhenius plots of the fluorescence anisotropy of diphenylhexatriene (r_{DPH}) in control and dexamethasone-treated rat proximal-small-intestinal Golgi membranes

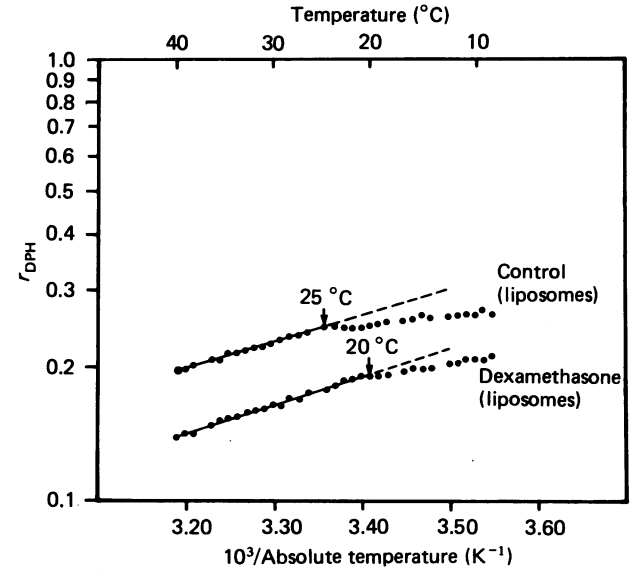

Fig. 2. Representative Arrhenius plots of the fluorescence anisotropy of diphenylhexatriene (r_{DPH}) in control and dexamethasone-treated liposomes prepared from the lipid extracts of rat proximal-small-intestinal Golgi membranes

by lower r_∞ values of DPH and r values of 2-AS and 12-AS respectively in treated membranes.

The effect of temperature on the DPH fluorescence anisotropy, r, in Golgi membranes and their liposomes prepared from the proximal small intestine of control and dexamethasone-treated rats are depicted by representative Arrhenius plots in Figs. 1 and 2. At each temperature tested the mean value of the fluorescence anisotropy, r, of the membranes and liposomes of dexamethsone-treated rats was significantly lower ($P < 0.005$) compared with respective control values. Changes in slopes of the Arrhenius plots, indicative of the lower critical temperatures of 'lipid thermotropic transitions' (Brasitus *et al.*, 1980) were observed in control and treated groups (Table 2) [the term 'lipid thermotropic transition' is used in a general sense to denote a thermally induced change in the physical state of the lipid of Golgi membranes; this change might involve order–disorder transitions of the liquid-crystalline gel type, lateral phase separations, lipid clusters or other mechanisms (Brasitus *et al.*, 1979)]. The break-points of control preparations (membranes and liposomes) were observed at 25–27 °C, whereas for preparations of the dexamethasone group they occurred at 20–21 °C. These findings are in agreement with previous studies by Chapman & Penkett (1966), which demonstrated that membranes with greater lipid fluidity were found to have lower transition temperatures. Additionally, the values of r of intact membranes of control and treated groups were consistently greater at each temperature point than those of their corresponding liposomes, a pattern previously observed in antipodal plasma membranes of rat enterocytes (Schachter & Shinitzky, 1977; Brasitus & Schachter, 1980; Brasitus *et al.*, 1980) and ascribed to the effects of the membrane proteins on lipid fluidity.

Table 2. Thermotropic transition temperatures of intact Golgi membranes and their liposomes of control and dexamethasone-treated rats

Values are means ± S.E.M. for four to six separate membrane preparations and their liposomes. *$P < 0.05$ or less compared with control values.

	Transition temp. (°C)	
Preparations	Control	Dexamethasone
Intact membranes	26.4±1.28	22.8±1.31*
Liposomes	23.8±1.77	20.0±1.00*

Lipid-compositional studies

Considerable evidence exists that differences in the lipid fluidity of model bilayers and natural membranes may be correlated with variations in their lipid and protein composition (Oldfield & Chapman, 1971; Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978). For example, a higher lipid fluidity has been correlated with lower ratios of cholesterol to phospholipid (mol/mol), sphingomyelin/phosphatidylcholine (mol/mol) and protein to lipid (w/w), as well as less saturated or shorter acyl chains in phospholipids (Hubbel & McConnell, 1971; Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978). In view of the differences in fluidity seen in control and treated small-intestinal Golgi membranes, it was of interest to examine and compare these parameters in membranes of both groups. As shown in Table 3, control and treated membranes possessed similar cholesterol/phospholipid (mol/mol), sphingomyelin/phosphatidylcholine (mol/mol) and protein/lipid (w/w) ratios. Dexamethasone-treated membranes, however, possessed a significantly lower saturation index and a higher double-bond index than their control counterparts (Table 3). Analysis of total fatty acids of these membranes (Table 4) revealed that Golgi

Table 3. Effect of dexamethasone administration to rats on the compositional parameters of small-intestinal Golgi membranes

Values are means ± S.E.M. for four to six preparations of Golgi membranes. The saturation index was calculated by dividing the total saturated acyl chains by the sum of each unsaturated chain multiplied by the number of double bonds. The double-bond index was calculated by adding each unsaturated chain multiplied by the number of double bonds/100. * $P < 0.05$ or less compared with control values.

Parameters	Control	Dexamethasone
Cholesterol/phospholipid (mol/mol) ratio	0.48 ± 0.04	0.41 ± 0.08
Sphingomyelin/phosphatidylcholine (mol/mol) ratio	0.41 ± 0.07	0.42 ± 0.11
Saturation index	0.58 ± 0.05	0.40 ± 0.04*
Double-bond index	1.01 ± 0.05	1.27 ± 0.06*
Protein/lipid (w/w) ratio	0.65 ± 0.04	0.60 ± 0.01

Table 4. Effect of dexamethasone administration to rats on composition of total fatty acids of rat proximal-small-intestinal Golgi membranes

Values are means ± S.E.M. of lipid extracts from four to six separate membrane preparations. * $P < 0.05$ or less compared with control values.

	Composition (% by mass)	
Fatty acid	Control	Dexamethasone
14:0	3.8 ± 0.3	0.5 ± 0.1*
14:1	2.4 ± 0.6	0.9 ± 0.1*
16:0	27.9 ± 0.3	27.0 ± 0.7
16:1	0.5 ± 0.2	0.7 ± 0.2
18:0	24.3 ± 1.2	20.0 ± 1.7*
18:1	11.3 ± 0.5	12.2 ± 0.2
18:2	17.1 ± 1.3	22.2 ± 1.1*
20:4	13.1 ± 0.9	16.6 ± 1.1*

membranes from treated-rats 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 their control counterparts. These latter alterations in fatty acids appeared to be responsible for the lower saturation index and higher double-bond index observed in Golgi membranes of dexamethasone-treated rats, and may at least partially explain the fluidity differences in these membranes (Shinitzky & Barenholz, 1978). In this regard, however, it is important to note that the effect of differing levels of unsaturation of fatty acids of phospholipids on the physiological properties of biological membranes is complex and difficult to ascertain (Stubbs, 1983; Stubbs & Smith, 1984). On the basis of a number of studies in model and biological membranes, it would appear that there is no simple relationship between the number of double bonds and their position with the physical parameters relating to rate and range of motion of the probes used in the present experiments (Stubbs & Smith, 1984). The relative percentages of individual

Table 5. Effect of dexamethasone administration to rats on composition of lipid extracts of proximal-small-intestinal Golgi membranes

	Composition [% (w/w) of total]	
Component	Control	Dexamethasone
Cholesterol	15.9 ± 0.7	14.2 ± 1.7
Cholesterol esters + triacylglycerols	6.6 ± 0.7	5.4 ± 0.9
Free fatty acids	11.9 ± 0.4	11.2 ± 1.2
Phosphatidylethanolamine	13.4 ± 0.7	12.4 ± 1.3
Phosphatidylcholine	26.4 ± 1.8	26.3 ± 2.5
Phosphatidylinositol	6.5 ± 0.7	8.5 ± 1.0
Phosphatidylserine	7.9 ± 2.5	10.5 ± 1.1
Sphingomyelin	10.7 ± 1.5	11.0 ± 2.5
Lysophosphatidylcholine	0.7 ± 0.2	0.5 ± 0.3

neutral lipids as well as phospholipids, however, remained unchanged in Golgi membrane preparations in control and treated rats (Table 5).

Effects of dexamethasone on enzymic activities

Prior studies from our laboratory and others (Brasitus & Keresztes, 1984; Brasitus & Dudeja, 1985; Brasitus, 1983; Wood *et al.*, 1985) have demonstrated a correlation between changes in the physical state of membrane lipids and alterations in membrane-bound enzymic activities. In this regard, UDP-galactosyl-lactosylceramide galactosyltransferase and CMP-*N*-acetylneuraminic acid: lactosylceramide sialyltransferase are Golgi membrane enzymes that utilize the common precursor, lactosylceramide, to produce globotriaosylceramide and haematoside (G_{M3}) respectively (Bouhours & Glickman, 1976, 1977). Since previous studies have suggested that the latter glycosphingolipids might be involved in rat small-intestinal differentiation and proliferation (Bouhours & Glickman, 1976, 1977), it was of interest to determine whether these enzymic activities could be modulated by the dexamethasone-induced alterations in the lipid fluidity and/or lipid composition of Golgi membranes seen in the present studies. As shown in Table 6, dexamethasone administration was found to significantly increase the activities of both these enzymes, thereby suggesting that dexamethasone increased the fluidity of Golgi membranes which, in turn, increased the enzyme activities.

In order to investigate further the possible relationship between lipid-fluidity alterations and changes in these enzyme activities, experiments were performed using benzyl alcohol and methyl alcohol. The former agent is a known fluidizer, whereas the latter alcohol is not (Gordon *et al.*, 1980). The differences in the effects of these alcohols on membrane fluidity appear to be related to their lipid/water partition coefficient (Fernandez *et al.*, 1984). In the present studies, benzyl alcohol (50 mM) significantly lowered the r values of DPH of Golgi membranes (control, 0.261 ± 0.003; treated, 0.237 ± 0.003; $n = 3$) [$P < 0.05$], whereas methyl alcohol did not (control, 0.260 ± 0.004; treated, 0.259 ± 0.003; $n = 3$). As shown in Table 6, benzyl alcohol (50 mM) also significantly increased the activity of UDP-galactosylactosyl-lactosylceramide galactosyltransferase

Table 6. Glycosphingolipid glycosyltransferase activities in rat proximal-small-intestinal Golgi membranes of control and dexamethasone-treated rats: effect of benzyl alcohol and methyl alcohol

Values are means ± S.E.M. for six separate preparations. * $P < 0.05$ or less compared with control values; ** $P < 0.05$ or less compared with control values with no addition; abbreviations used: BzOH, benzyl alcohol; MeOH, methyl alcohol.

Enzyme	Additions	Activity (nmol/mg of protein)	
		Control	Dexamethasone
CMP-acetylneuraminic acid:lactosylceramide sialytransferase	None	27.2 ± 1.2	63.0 ± 1.4*
	50 mM-BzOH	25.0 ± 1.6	–
	50 mM-MeOH	28.4 ± 1.2	–
UDP-galactosyl-lactosylceramide galactosyltransferase	None	32.7 ± 1.4	50.1 ± 3.5*
	50 mM-BzOH	39.3 ± 1.2**	–
	50 mM-MeOH	34.7 ± 1.7	–

(approx. 20%), but had no effect on CMP-acetylneuraminic acid:lactosylceramide sialyltransferase activity in rat proximal-small-intestinal Golgi membranes. Methyl alcohol (50 mM) had no effect on either of these enzyme activities.

DISCUSSION

Previous studies (Johnston & Melnykovych, 1980; Boullier *et al.*, 1982; Kapitulnik *et al.*, 1986; Brasitus *et al.*, 1987; Dudeja *et al.*, 1987) have shown that dexamethasone increased the lipid fluidity of plasma membranes or intracellular membranes in various cell types. Thus incubation of HeLa cells with dexamethasone for 24 h was previously found to lower the steady-state fluorescence polarization of DPH in intact HeLa-cell membranes and isolated plasma membranes by Johnston & Melnykovych (1980) and Boullier *et al.* (1982). The latter investigators also demonstrated that this glucocorticoid increased the lateral diffusion of the fluorescent lipid analogue 3,3′-dioctadecylindocarbocyanine iodine in HeLa-cell membranes using a fluorescence-photobleaching-recovery technique (Boullier *et al.*, 1982). Dexamethasone, administered to pregnant rats during the last week of pregnancy, was also found to decrease DPH anistropy values in fetal-rat liver microsomal membranes (Kapitulnik *et al.*, 1986). Additionally, our laboratory has recently demonstrated that administration of this glucocorticoid to rats increased the fluidity of rat proximal-small-intestinal brush-border membranes (Brasitus *et al.*, 1987) and rat distal-colonic apical plasma membranes (Dudeja *et al.*, 1987). In agreement with these previous findings, the present results demonstrate, for the first time, that dexamethasone treatment also increased the 'static' and 'dynamic' components of fluidity of rat proximal-small-intestinal Golgi membranes, as assessed by r_∞ values of DPH and r values of 2-AS and 12-AS respectively.

This increase in fluidity of Golgi membranes prepared from treated animals, moreover, appeared to be due to alterations in the levels of stearic, linoleic and arachidonic acids, thereby resulting in an increased double-bond index and a decreased saturation index in treated membranes. In this regard an increase in polyunsaturated fatty acids after dexamethasone administration was also previously noted in fetal-rat hepatic microsomal membranes (Kapitulnik *et al.*, 1986) and rat proximal-small-intestinal brush-border membranes (Brasitus *et al.*, 1987*b*). In the latter studies, our laboratory demonstrated that increases in the activities of lysophosphatidylcholine acyltransferase, with linoleoyl-CoA and arachidonyl-CoA, were significantly increased by 4 days of dexamethasone administration in cellular homogenates of the rat proximal small intestine. Although these activities were not measured in the present experiments, since the same dexamethasone treatment regimen (100 μg/100 g body wt./day for 4 days) was used in both studies, it would appear reasonable to ascribe the present fatty acid changes to alterations in these acyltransferase activities.

Dexamethasone treatment also increased the specific activities of CMP-acetylneuraminic acid:lactosylceramide sialyltransferase and UDP-galactosylceramide galactosyltransferase in Golgi membranes. In view of the results of previous studies by our laboratory (Brasitus *et al.*, 1979; Brasitus *et al.*, 1980; Brasitus, 1983), which demonstrated a correlation between alterations in the physical state of membrane lipids and changes in a number of different enzyme activities, it initially appeared that the increase in Golgi membrane fluidity induced by dexamethasone was responsible for increases in both glycosphingolipid glycosyltransferase activities. Further studies with benzyl alcohol, an agent which fluidized the Golgi membranes, however, demonstrated that the activity of UDP-galactosyl-lactosylceramide galactosyltransferase, but not CMP-acetylneuraminic acid:lactosylceramide sialyltransferase, increased concomitantly with benzyl alcohol-induced increases in Golgi membrane fluidity. These findings are in basic agreement with those from previous studies (Mitranic *et al.*, 1982) in rat hepatic Golgi membranes, which demonstrated that various membrane perturbing agents, including benzyl alcohol, had different effects on the activities of several glycosyltransferases. These investigators, moreover, showed that benzyl alcohol did not alter the activity of milk galactosyltransferase incorporated into dimyristoyl or dipalmitoyl phosphatidylcholine liposomes, but did show a complex effect on the activity of this enzyme incorporated into distearoyl phosphatidylcholine liposomes, i.e., stimulation of activity at low concentrations, followed by inhibition at higher concentrations of this agent. On the basis of these observations they suggested that the different effects of benzyl alcohol on rat hepatic Golgi glycosyltransferase activities might be secondary

to variations in the lipid environment of these enzymic activities. This could also serve as a possible explanation for the present results seen with benzyl alcohol on the glycolipid glycosyltransferases examined in rat proximal-small-intestinal Golgi membranes. The mechanism(s) underlying the alterations in the activities of the various glycosphingolipid-biosynthetic enzymes seen in Golgi membranes of dexamethasone-treated animals, therefore, appear complex and are unclear at this time. The present studies do, however, suggest that at least the activity of UDP-galactosyl-lactosylceramide galactosyltransferase, but not CMP-acetylneuraminic acid:lactosylceramide sialyltransferase, may be influenced by dexamethasone-induced changes in membrane fluidity.

Regardless of the mechanism(s) involved, however, administration of dexamethasone did increase the specific activities of these two glycosphingolipid glycosyltransferases. As noted above, previous studies on the rat small intestine have suggested that ganglioside G_{M3} and globotriaosylceramide, the products of these enzymes, may be involved in cellular differentiation and proliferation in this organ (Bouhours & Glickman, 1976, 1977). Addition of exogenous G_{M3} to several different cell lines has also been shown to inhibit cell growth (Bremer & Hakomori, 1982). Moreover, recent investigations (Bremer *et al.*, 1984) have demonstrated that the levels of several gangliosides, including G_{M3}, appeared to modulate the affinity of platelet-derived growth factor for its receptors by affecting the degree of receptor tyrosine phosphorylation in mouse Swiss 3T3 cells. Further studies along these lines should elucidate the mechanism(s) responsible for the dexamethasone-induced alterations in these glycosphingolipid glycosyltransferase activities as well as clarify their functional significance in rat proximal-small-intestinal Golgi membranes.

We thank Ms. Lynn Nelson for her excellent secretarial assistance. This investigation was supported by U.S. Public Health Grant no. CA 36745 awarded by the National Cancer Institute (N.C.I.), Department of Health and Human Services. T.A.B. is the recipient of a Merit Award from the N.C.I./National Institutes of Health.

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
Binder, H. J. (1978) Gastroenterology **75**, 212–217
Bouhours, J. F. & Glickman, R. M. (1976) Biochim. Biophys. Acta **441**, 123–133
Bouhours, J. F. & Glickman, R. M. (1977) Biochim. Biophys. Acta **487**, 51–60
Boullier, J. A., Melnykovych, G. & Barias, B. G. (1982) Biochim. Biophys. Acta **692**, 278–286
Brasitus, T. A. (1983) Biochim. Biophys. Acta **728**, 20–30
Brasitus, T. A. & Dudeja, P. K. (1985) J. Biol. Chem. **260**, 12405–12409
Brasitus, T. A. & Keresztes, R. S. (1984) Biochim. Biophys. Acta **773**, 290–300
Brasitus, T. A. & Schachter, D. (1980) Biochemistry **19**, 2763–2769
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., Dudeja, P. K., Eby, B. & Lau, K. (1986) J. Biol. Chem. **26**, 16404–16409
Brasitus, T. A., Dudeja, P. K., Dahiya, R. & Halline, A. (1987) Biochem. J. **248**, 455–461
Brasitus, T. A., Dahiya, R. & Dudeja, P. K. (1988) Biochim. Biophys. Acta **958**, 218–226
Bremer, E. G. & Hakomori, S. I. (1982) Biochem. Biophys. Res. Commun. **106**, 711–714
Bremer, E. G., Hakomori, S. I., Bowen-Pope, D. F., Raines, E. & Ross, R. (1984) J. Biol. Chem. **259**, 6818–6825
Burzak, J. D., Soltysiak, R. M. & Swelley, C. C. (1984) J. Lipid Res. **25**, 1541–1547
Chapman, D. & Penkett, S. A. (1966) Nature (London) **211**, 1304–1305
Charney, A. N., Kinsey, M. D., Meyers, L., Gianella, R. A. & Gots, R. E. (1975) J. Clin. Invest. **56**, 6353–6360
Cogan, U. & Schachter, D. (1981) Biochemistry **20**, 6396–6403
Dahiya, R. & Brasitus, T. A. (1987) Biochim. Biophys. Acta **922**, 118–124
Dahiya, R., Dudeja, P. K. & Brasitus, T. A. (1987) Cancer Res. **47**, 1031–1035
Daniels, V. G., Hardy, R. N., Malinowska, K. W. & Nantha-Nielz, P. W. (1973) J. Physiol. (London) **229**, 681–695
Dudeja, P. K., Foster, E. S. & Brasitus, T. A. (1987) Biochim. Biophys. Acta **905**, 485–493
Farquhar, M. G. & Palade, G. E. (1981) J. Cell. Biol. **91**, 77S–103S
Fernandez, Y. L., Biogegrain, R. M., Chambon-Gross, C. D. & Mitjavily, S. E. (1984) Biochim. Biophys. Acta **770**, 171–177
Field, M. (1978) Gastroenterology **75**, 317–319
Fleischer, B. J. & Fleischer, S. (1970) Biochim. Biophys. Acta **219**, 301–319
Folch, J., Lees, M. J. & Sloane-Stanley, G. A. (1957) J. Biol. Chem. **226**, 497–509
Freedman, R. A., Weiser, M. M. & Isselbacher, K. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 3612–3616
Gartner, S. L. & Vahouny, M. G. (1972) Am. J. Physiol. **222**, 1121–1124
Gordon, L. M., Sauerheber, R. D., Esgate, J. A., Dipple, I., Marchmont, R. J. & Houslay, M. D. (1980) J. Biol. Chem. **225**, 4519–4527
Henning, S. J. & Sims, M. (1979) Endocrinology (Baltimore) **104**, 1158–1164
Heyn, M. P. (1979) FEBS Lett. **108**, 520–527
Higgins, J. A. & Fieldsend, J. K. (1987) J. Lipid Res. **28**, 268–278
Higgins, J. A. & Hutson, J. L. (1984) J. Lipid Res. **25**, 1295–1305
Hubbel, 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
Jones, A. L., Ruderman, N. B. & Herrera, M. G. (1967) J. Lipid Res. **8**, 429–446
Kapitulnik, 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
Kaufman, B. & Basu, S. (1966) Methods Enzymol. **8**, 365–368
Keenan, T. W., Morre, D. J. & Basu, S. (1974) J. Biol. Chem. **249**, 310–315
Kim, Y. S., Perdomo, J. & Whitehead, J. S. (1972) J. Clin. Invest. **51**, 2024–2032
Lackowicz, J. R., Prendergast, F. G. & Hogen, D. (1979) Biochemistry **18**, 508–519

Lebenthel, E., Sunshine, P. & Kretchmer, N. (1972) J. Clin. Invest. **51**, 1244–1250
Lentze, M. J., Colony, P. C. & Trier, J. S. (1985) Am. J. Physiol. **249**, G58–G65
Lowry, O. H., Rosebrough, N., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Marnane, W. G., Tai, Y. H., Decker, R. A., Boedeker, E. C., Charney, A. N. & Donowitz, M. (1981) Gastroenterology **81**, 91–100
Meneely, R. & Ghishan, F. K. (1982) Pediatr. Res. **16**, 776–778
Mitranic, M. M., Boggs, J. M. & Moscarello, M. A. (1982) Biochim. Biophys. Acta **693**, 75–84
Oldfield, E. & Chapman, D. (1971) Biochem. Biophys. Res. Commun. **43**, 610–616
Podolsky, D. K. & Weiser, M. M. (1975) Biochem. Biophys. Res. Commun. **65**, 543–551
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., Watkins, J. B., Ling, S. C., Fayer, J. C. & Mone, M. (1986) Biochim. Biophys. Acta **860**, 411–419
Scott, J., Batt, R. M., Maddison, Y. E. & Peter, T. J. (1981) Am. J. Physiol. **241**, G306–G132
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, N. (1976) Biochim. Biophys. Acta **433**, 133–149
Stubbs, C. D. (1983) Essays Biochem. **19**, 1–39
Stubbs, C. D. & Smith, A. D. (1984) Biochim. Biophys. Acta **779**, 89–137
Van Blitterswijk, W. J., Van Hoeven, R. P. & Van der Meer, B. W. (1981) Biochim. Biophys. Acta **644**, 323–332
Weiser, M. (1973) J. Biol. Chem. **248**, 2536–2541
Wood, P. A., McBride, M. R., Baker, H. J. & Christian, S. T. (1985) J. Neurochem. **44**, 947–956
Yusefet, H. K. M., Pohlentz, G., Schwarzmann, G. & Sandhoff, K. (1983) Eur. J. Biochem. **134**, 47–54
Zlatkis, A., Jak, B. & Boyle, A. J. (1953) Lab. Clin. Med. **41**, 486–492

Received 4 December 1987/25February 1988; accepted 6 April 1988