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Glucocorticoid-Mediated Alteration of Fluidity of Brush Border Membrane in Rat Small Intestine

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ABSTRACT. The normal biophysical properties of the maturing mammalian enterocyte membrane are poorly understood. While the effects of glucocorticoids on maturation of intestinal enzyme function has been intensively investigated, their effects on membrane biophysical properties are not known. We used 1,6-diphenyl-1,3,5-hexatriene as a probe in fluorescence anisotropy studies to determine the fluidity of rat brush border membrane. Maturational changes and the effects of glucocorticoids administered antenatally or postnatally were determined. Fluorescence anisotropy values for 1,6-diphenyl-1,3,5-hexatriene in mature brush border membranes were higher than those values in membranes obtained from younger animals reflecting a less fluid membrane. Glucocorticoids administered to suckling rats increased the anisotropy values of 1,6-diphenyl-1,3,5-hexatriene in the membranes compared to saline-administered littermates. The anisotropy of intestinal brush border membrane was also increased in fetal rats whose mothers received dexamethasone. These alterations may relate to protein-binding properties and permeability characteristics of the enterocyte membrane. (*Pediatr Res* 20: 79–82, 1986)

Abbreviation

DPH, 1,6-diphenyl-1,3,5-hexatriene

Little is known about the biophysical properties of intestinal membranes during maturation or about the effects of glucocorticoids on the development of these membranes. In the past decade, methods have been developed to isolate brush border membranes of intestinal enterocytes (1, 2) and to characterize their biophysical properties using fluorescence polarization (3). Because alterations in membrane biophysical properties, such as fluidity, may be related to altered protein binding of these membranes (in addition to changing permeability), it is important to understand the normal ontogeny of these membrane properties and the effects of pharmacologic and dietary manipulation.

Glucocorticoids are known to accelerate intestinal disaccharidases in both fetal and suckling rat intestine (4). Information about the effects of agents such as glucocorticoids on the biophysical properties of intestinal brush border membranes is lacking and may be important in understanding how they relate to maturation of the intestinal mucosal barrier. Accordingly, the purpose of this study was to investigate: 1) the biophysical characteristics of normally developing enterocyte membrane and 2) the effects of exogenously administered glucocorticoids on these membranes' characteristics.

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MATERIALS AND METHODS

The study used Sprague-Dawley rats ranging in age from fetal life to young adulthood: 22-day-old fetuses and 5, 10, 19, and 30 days of postnatal age (n = 6, 2, 4, 3, 2 rats, respectively). These animals were used to study the normal changes that occur in the fluidity of intestinal brush border membrane during development. The pharmacologic effects of glucocorticoids administered both ante- and postnatally were studied to determine whether a maturational effect could be induced in the biophysical properties of these membranes. Hydrocortisone succinate, 50 mg/kg, was administered three times daily to suckling rats (n = 4) between 10 and 13 days of age. Control littermates (n = 4) were given equivalent volumes of 0.9% saline subcutaneously. The rats were killed on day 14. Dexamethasone, 0.4 mg/kg, was administered to pregnant rats intraperitoneally three times daily between days 20–22 of gestation. Pregnant control rats (n = 4) were given equal volumes of 0.9% saline on the same dosage schedule. Fetuses were delivered at 22 days of gestation by cesearean by cesearean section and killed by decapitation for intestinal analysis.

Intestines of the rats used for the developmental and infant glucocorticoid studies were gently flushed with 0.9% saline at 0° C. Brush border membranes were isolated by the Malathi modification (1) of the Schmitz method (2). A 1% homogenate was made in 50 mM mannitol-2 mM Tris solution (pH 7.1). Fetal intestines, which could not be flushed because of their small size, were individually homogenized to 1% in the same buffer. Purification of brush border membrane in all samples was validated by an increased small intestinal maltase activity per milligram of protein over that from the original homogenate. Maltase activity was measured by the method of Tsuboi *et al.* (5) and expressed as moles per minute per milligram of protein. Protein in the homogenate and brush border membrane fractions was measured by the method of Lowry *et al.* (6). Enhancement of maltase activity in brush border membrane over that from original homogenate ([specific activity of purified membranes] ÷ [specific activity of homogenate]) is shown in Table 1.

Membrane fluidity studies were performed using a stock solution of 0.001 M DPH (7) in tetrahydrofuran. On each day of the fluorescence studies, an aliquot of this stock solution was diluted to 10 μM using 0.02 M Na_2HPO_4, pH 7.4, and the suspension was stirred for 2 h. An aliquot of brush border membranes containing greater than 5 μg of protein was added to 2.4 ml of the diluted DPH in buffer and incubated at 37° C. After the 2 h, equilibrations of DPH was complete since there was no further increase in the fluorescence intensity. Some of the fetal membranes were pooled (from one to six fetuses per pool) into their individual groups in order to obtain greater than 5 μg protein for fluorescence polarization measurements. The

Table 1. *Increase in maltase activity in brush border membrane preparations*

Age (day)	*n**	Treatment	Increase mean ± SD maltase activity (mol/min/min)
5	4	0	9.63 ± 1.58
10	4	0	11.34 ± 3.37
19	4	0	16.65 ± 4.19
25	3	0	15.96 ± 1.33
30	2	0	23.90 ± 8.07
22 Fetus†	6	Dexamethasone	6.28 ± 1.99
22 Fetus†	6	Saline	7.24 ± 1.44
14	4	Hydrocortisone	11.63 ± 0.93
14	4	Saline	10.78 ± 0.76

* Number of pools (one to six animals per pool).

† Both fetuses were 22 days' gestational age.

anisotropy values of the brush border membranes from each pool were counted as only one value in the statistical analyses.

Fluorescence polarization of these samples was measured with an SLM 4800 subnanosecond spectrofluorometer (SLM Instruments, Inc., Urbana, IL), interfaced with a PDP/LSI-11/2 computer. Anisotropy (r) was calculated by the following equation after measuring the intensities of emitted light that were either vertically ($I_\parallel$) or perpendicularly ($I_\perp$) oriented with respect to the direction of the exciting light (7, 8).

$$(r) = \frac{(I_\parallel - I_\perp)}{(I_\parallel + 2I_\perp)}$$

Fluorescence lifetimes were measured using phase modulation techniques with a modulation frequency of 30 MHz, with the excitation polarizer set to 53°. Analysis of variance showed no significant differences in excited state lifetimes among the comparison groups. Fluorescence intensities ($I_\parallel + 2I_\perp$) were similarly compared and no differences were found among the groups that were compared.

Emission intensities for each sample were measured and averaged 10 times at 4° C intervals from 40 to 12° C using a water bath. Excitation wavelength was set at 365 nm and emission wavelength at 410 nm.

Slopes and y-intercepts were determined using linear regression analysis on the individual anisotropy versus temperature curves. Statistical analysis for the anisotropy determinations consisted of analysis of variance among the resulting fetal, 5-, 10-, 19-, and 30-day-old slopes and y-intercepts with individual controls using Dunnett's procedure with the fetal membranes as controls. Student's t test was used to compare glucocorticoid versus saline administered anisotropy curve slopes and y-intercepts. Maltase activity was compared in control versus glucocorticoid treated animals. Student t tests were used for these comparisons.

RESULTS

Table 1 shows the enrichment of maltase activity per milligram of protein in brush border membrane over that from the original homogenate. No differences were found in maltase increases in the fetal or 14-day-old steroid-treated experimental rats *versus* the control rats.

As demonstrated in previous studies (4), maltase activity increased during maturation. This normal increase was accelerated by glucocorticoid administration. In these same rats, changes in membrane lipid phase behavior were also demonstrated (Table 2) between animals at different stages of development. Membrane fluidity determinations may be expressed using a lipid order parameter(s) (10). This lipid order parameter is largely dependent on the slow decaying static component of the fluorescence anisotropy, r_∞, which can be related to the anisotropy, r_s, by the formula:

$$r_\infty = 4/3r_s - 0.1, \qquad \text{If } 0.13 < r_s < 0.28$$

Since the majority of our anisotropy (r_s) values at temperature >25° C and in the younger animals were below the range for which this semiempirical relation equation can be used ($r_s < 0.13$), lipid order parameters were not calculated. Other authors (10) have commented on the fact that DPH is not completely isotropic, hence, microviscosity values cannot strictly be used. Because of these reasons, anisotropy values are used here since no assumptions are made in their calculation. Furthermore, since the fluorescence lifetimes were similar for all samples, the fluorescence anisotropy can be used as a reliable comparative index of the rotational motion of DPH and the fluidity of the membrane.

As the temperature was changed from 40 to 12° C, the anisotropy or rotational motion of DPH embedded in the membrane decreased as evidenced by the consistent slope of the temperature versus anisotropy curves. Both the slopes and y-intercepts were used as indices of the degree of rotational motion of DPH. For example, the y-intercept values or initial anisotropy values of membranes from older animals were significantly higher than those of membranes from younger animals. Furthermore, consistently more negative slopes reflecting a more ordered lipid phase behavior in the more mature animals were observed. The anisotropy of the brush border membranes of dexamethasone-exposed fetuses was higher than that in the saline-administered controls (Table 3). The anisotropies of membranes from 14-day-old rats administered hydrocortisone were also higher than those given saline (Table 3). Dexamethasone administered to pregnant rats also increased the fetal intestinal maltase activity (Fig. 1). This is an effect which has been described previously (4) and is being shown here to validate the fact that glucocorticoids used traversed the placenta into the fetal circulation causing known effects.

Table 2. *Comparison of rat microvillus membranes anisotropy during development*

Age (day)	(*n*)	Slope ($\times 10^{-3}$) of temperature (°C) *vs* anisotropy curve (40–12° C)	y-intercepts of temperature (°C) *vs* anisotropy curve (40–12° C)
22 fetus	(6)	−0.678 ± 0.185	0.1254 ± 0.0058
5	(2)	−0.960 ± 0.034	0.1440 ± 0.0020†
10	(4)	−1.280 ± 0.454†	0.1486 ± 0.0117‡
19	(4)	−0.996 ± 0.248	0.1548 ± 0.0090‡
30	(2)	−1.441 ± 0.028†	0.1461 ± 0.0007†

* Values of slopes and y-intercepts are means ± SD. ANOVA difference among slopes significant at $p < 0.05$ ($F = 4.35$) and among y-intercepts at $p < 0.01$ ($F = 9.36$).

Individual contrasts were done using Dunnet's procedure with the fetal animals as controls: †$p < 0.05$ and ‡$p < 0.01$.

Table 3. *Effects of glucocorticoids administered antenatally or postnatally on rat microvillus membrane anisotropy**

Age (day)	(*n*)	Treatment	Slope $\times 10^{-3}$ temperature (°C) *vs* anisotropy curve (40–12°C)	y-intercepts of temperature (°C) *vs* anisotropy curve (40–12° C)
22 fetus	(5)	Saline	−0.678 ± 0.185	0.1253 ± 0.0058
22 fetus	(4)	Dexamethasone	−1.095 ± 0.189*	0.1422 ± 0.0058*
14	(4)	Saline	−0.996 ± 0.160	0.1444 ± 0.0055
14	(4)	Hydrocortisone	−1.162 ± 0.286	0.1570 ± 0.0096†

* Values of slopes and y-intercepts are means ± SD.

Significance of difference between means for glucocorticoid *versus* saline treated animals *$p < 0.005$ and †$p < 0.05$ using Student's t-test.

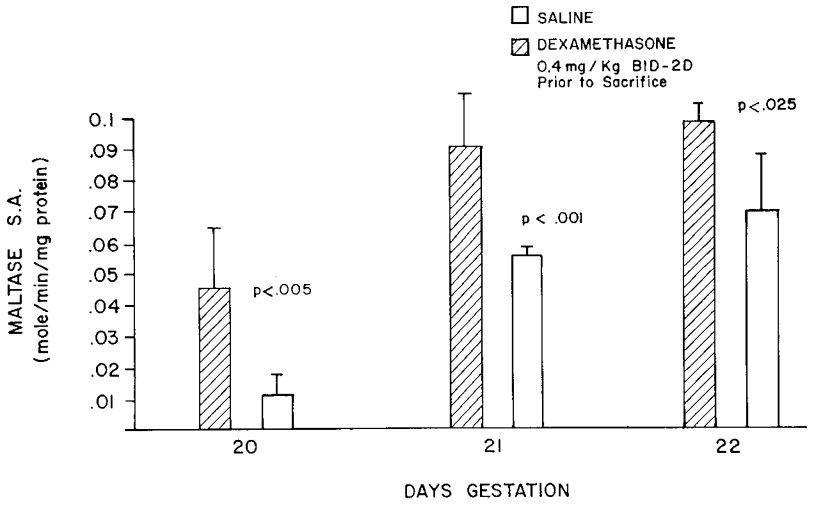

Fig. 1. Effect on fetal intestinal maltase of dexamethasone *versus* saline administered to pregnant rats for 2 days prior to caesarean section.

DISCUSSION

The intestinal brush border membrane performs transport and enzymatic functions that are essential for normal digestion and absorption. It also acts as a protective barrier against noxious agents and a binding site for immunoglobulins. Our fluorescence polarization studies demonstrate alterations in biophysical properties of the intestinal membrane generated by normal maturation and by the exogenous administration of glucocorticoids to fetal and infant rats. The membranes from more mature rats demonstrated greater anisotropy (lower fluidity) than did those from younger animals. The fetal and infant rats given glucocorticoids similarly demonstrated higher anisotropies than their saline-administered controls, suggesting an accelerated maturation of their brush border membrane.

Developmental alterations in the intestinal microvillus membrane have been previously reported (11–13). Schwartz *et al.* (11), using fluorescence polarization techniques with both rabbit and rat microvillus membranes, demonstrated a significant increase in anisotropy from infancy to adulthood. This occurred despite a decrement in jejunal microvillus cholesterol/phospholipid molar ratio. Pang *et al.* (12) used electron spin resonance and spin label methods to investigate the biophysical properties of microvillus membrane from the small intestine of adult and newborn rabbits. The spin label environment of the newborn microvillus membranes appeared to be in a more disordered environment than the adult. Along with the differences in membrane organization, an increased binding of cholera toxin was observed in the newborn microvillus membrane suggesting that differences in membrane organization might, in part, account for the increased attachment and penetration of macromolecules noted during the perinatal period. Brasitus *et al.* (13) found progressive decreases in fluidity of rat intestinal membranes analyzed at 6, 17, and 117 wk of age. Israel *et al.* (14) reported that exogenously administered thyroxine decreases the fluidity of rat microvillus membranes in addition to decreasing binding of immunoglobulins. In a more recent report, Pang *et al.* (15), using electron spin resonance techniques, demonstrated that *in utero* exposure to cortisone administered exogenously to pregnant rats will accelerate maturation of the microvillus surface of fetus enterocytes.

Our studies confirmed the findings of Schwartz *et al.* (11) Pang *et al.* (12) and Brasitus *et al.* (13) regarding the maturational changes that occur in microvillus membrane anisotropy during normal development. We have also confirmed, using fluorescence polarization, the recently reported finding (15) of accelerated maturation of fetal microvillus membrane by administration of glucocorticoids to the mother. In addition, our results indicate that these maturational changes in microvillus membrane fluidity can also be induced postnatally by the administration of glucocorticoids.

In these studies, microvillus brush border membranes could be purified more successfully with increasing gestational age (Table 1). It is possible that the increased purity of the membranes in the older animals may have contributed to the altered anisotropy values with increasing age. However, the purity of brush border membrane in the fetal and infant groups given glucocorticoids *versus* saline were approximately equal. Thus, differences in their anisotropies could not be attributed to differences in membrane purity between control *versus* experimental, glucocorticoid-administered groups.

From our results and those reported by others, we propose that accelerated maturation of the brush border membrane by glucocorticoids may result in a less fluid membrane that provides greater resistance against potentially toxic agents. Exogenously administered glucocorticoids cause accelerated maturation of intestinal microvillus membranes in both fetal and infant rats. These alterations may relate to binding and permeability properties of the membrane and may have implications in prophylaxis and/or therapy of certain intestinal disease states where the integrity of the mucosal barrier is affected.

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