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Maturational Effects of Glucocorticoids on Neonatal Brush-Border Membrane Phosphate Transport

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ABSTRACT. Previous studies have implicated glucocorticoids as an important factor in the postnatal maturational increase in proximal tubule volume absorption, Na^+/H^+ antiporter, $Na(HCO_3)_3$ symporter, and Na^+-K^+-ATPase activity. The present study examined whether glucocorticoids are also a potentially important factor in the maturational decrease in proximal tubule phosphate transport. Renal BBMs were prepared from neonatal rabbits who received dexamethasone (10 μg/100 g body weight) or vehicle. Brush-border membrane vesicles from dexamethasone-treated neonates had a lower rate of Na-phosphate cotransport than controls (50.8 ± 3.6 *versus* 29.2 ± 2.6 pmol $^{32}P_i$/10 s/mg protein, $p < 0.001$). This decrease was due to a decrease in the V_{max} with no change in the affinity of the transporter for phosphate. The dexamethasone-induced decrease in BBM Na-phosphate transport was not due to a reduction in transporters as assayed by phosphate-protectable Na-dependent equilibrium binding of phosphonoformic acid. Dexamethasone treatment caused an increase in the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene and trimethylammonium-1,6-diphenyl-1,3,5-hexatriene (*i.e.* a decrease in membrane fluidity). Brush-border membranes from dexamethasone-treated neonates had a decrease in sphingomyelin and an increase in phosphatidylcholine and phosphatidylinositol content but no change in cholesterol or total phospholipid content. These data are consistent with glucocorticoids playing a role in the postnatal maturational decrease in proximal tubule phosphate transport by altering membrane characteristics. (*Pediatr Res* 35: 474–478, 1994)

Abbreviations

BBM, brush-border membrane
DPH, 1,6-diphenyl-1,3,5-hexatriene
TMA, trimethylammonium
PFA, phosphonoformic acid
BLM, basolateral membrane
P_i, phosphate

The neonatal proximal tubule has a lower rate of volume reabsorption than the proximal tubule from adult animals (1–3). This overall lower rate of solute transport by the neonatal proximal tubule is due, in part, to a lower rate of glucose transport (2–4) and bicarbonate transport (2, 3) by the immature segment. The rate of apical sodium-glucose cotransport (4), Na^+/H^+ antiporter activity (5–7) and H^+-ATPase activity (6), and basolateral Na^+-K^+-ATPase (1, 8–10) and $Na(HCO_3)_3$ symporter (5) activity are all significantly less than that in the mature proximal tubule.

Substantial evidence exists that unlike the proximal tubule transport processes described above, renal phosphate transport is higher in neonates than adult animals. Fractional reabsorption of phosphate is higher in infants than in older children (11). Maximal tubular reabsorption of phosphate per volume glomerular filtration rate is higher in neonatal rats than in adult rats, a difference that persists after thyroparathyroidectomy (12–14). There is also direct evidence that the higher rate of phosphate reabsorption by the neonate is due in part to a higher intrinsic rate of phosphate reabsorption by the neonatal kidney (15, 16). Neonatal guinea pig kidneys perfused *in vitro* had a higher maximal tubular reabsorption of phosphate per glomerular filtration rate than adult kidneys (15). In addition, neonatal guinea pig BBM vesicles had a higher V_{max} for phosphate uptake than those from adult animals (16).

The plasma glucocorticoid concentration rises in the perinatal period (17–19). This rise in glucocorticoid concentration likely plays an important role in the postnatal maturation of the proximal tubule (1–3, 7, 8). Neonates given glucocorticoids have an increase in proximal tubule Na^+-K^+-ATPase activity (1, 3, 8, 9). Fetal rabbits whose mothers were given 50 μg/kg of betamethasone daily for 2 d before delivery had a higher rate of renal BBM vesicle Na^+/H^+ antiporter activity and renal Na^+-K^+-ATPase activity than neonatal vehicle-treated controls (7). Using a similar protocol, we have found that proximal convoluted tubules from neonates whose mothers were treated with dexamethasone 2 d before delivery had a higher rate of volume absorption, bicarbonate transport, a more lumen-negative transepithelial potential difference, and higher rates of apical Na^+/H^+ antiporter and basolateral $Na(HCO_3)_3$ symporter activity than control neonates (2). The purpose of the present study was to examine whether glucocorticoids potentially play a role in the maturational decrease in renal phosphate transport.

MATERIALS AND METHODS

Animals. New Zealand White rabbit pregnant does were housed at our institution for at least 6 d before their expected date of delivery. Neonatal rabbits were cared for by their mothers. At 3–5 d of life they received either daily s.c. injections of dexamethasone (10 μg/100 g body weight) or vehicle for 4 d, including a dose 2 h before they were killed. Dexamethasone was dissolved in 150 mmol/L NaCl and 2 mmol/L K_2HPO_4 (pH, 7.5). Blood and urine samples were collected for measurement of serum and urine creatinine and phosphate for determination of fractional excretion of phosphate.

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BBM vesicle isolation. Control and dexamethasone-treated neonatal rabbits were killed, and the kidneys were rapidly removed and placed in an ice-cold homogenizing buffer containing (in mmol/L) 150 KCl, 16 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.50, with Tris. The cortex was isolated and homogenized with a Teflon-glass Potter Eljevhem homogenizer. BBM vesicles were then isolated by differential centrifugation and magnesium precipitation as previously described (4, 7, 20, 21). The final BBM vesicle fraction was resuspended in the above buffer at an approximate concentration of 10 mg BBM protein/mL for equilibrium binding studies. For transport studies, BBM were resuspended in a buffer containing (in mmol/L) 300 mannitol, 16 HEPES, titrated to pH 7.5 with Tris. Protein was measured by the method of Lowry *et al.* (22) with crystalline BSA as the standard. To minimize the potential day-to-day variation in the BBM isolation procedure, we isolated BBM from the kidneys of control and dexamethasone-treated neonatal rabbits simultaneously each day. For each BBM sample the kidneys from two rabbits were pooled ($n = 1$).

BBM enzyme activity measurements. The purity of each BBM preparation was determined by measurement of membrane-specific enzyme activity, including alkaline phosphatase, γ-glutamyltransferase, and leucine aminopeptidase (BBM bound), and Na^+-K^+-ATPase (BLM bound) in cortical homogenate and BBM fractions using a Gilford Response spectrophotometer (Ciba-Corning, Oberlin, OH) equipped with kinetic and temperature control unit, as we have previously described (20, 21). Alkaline phosphatase activity was measured by a kinetic assay monitoring the production of *p*-nitrophenolate from *p*-nitrophenyl phosphate at 405 nm and 37°C (23). Leucine aminopeptidase activity was measured by a kinetic assay that monitored the conversion of L-leucine-*p*-nitroanilide at 380 nm and 37°C (24). γ-Glutamyltransferase activity was measured by a kinetic assay monitoring the conversion of L-γ-glutamyl-*p*-nitroanilide at 405 nm and 37°C (25). Na^+-K^+-ATPase activity was measured by a kinetic assay system coupling ATP hydrolysis to pyruvate kinase and lactate dehydrogenase and monitoring the use of NADH at 340 nm and 37°C (26). Enzyme activities are expressed as picomoles per minute per milligram cortical homogenate or BBM protein. Enrichment (specific activity in BBM fraction/specific activity in homogenate) was determined in each BBM preparation.

BBM transport activity measurements. Transport measurements were performed in freshly isolated BBM vesicles by radiotracer uptake followed by rapid Millipore filtration. All uptake measurements were performed in triplicate, and the uptake was calculated on the basis of specific activity determined in each experiment and expressed as picomoles of solute/time interval/milligram BBM protein.

To measure Na^+ gradient-dependent ^{32}P uptake (Na-P_i cotransport), 10 μL of BBM preloaded in an intravesicular buffer of 300 mmol/L mannitol, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50, was vortex mixed at 25°C with 40 μL of an extravesicular uptake buffer of 150 mmol/L NaCl, 25 μmol/L $K_2H^{32}PO_4$, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50. Uptake after 10 s (representing initial linear rate) was terminated by an ice-cold stop solution that consisted of 135 mmol/L NaCl, 10 mmol/L Na arsenate, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50. To determine the Na^+-independent (*i.e.* diffusive) P_i uptake, 150 mmol/L NaCl was replaced with 150 mmol/L choline chloride. To determine whether the effect of dexamethasone on Na-P_i cotransport was due to a difference in V_{max} or the Km for P_i, we measured Na-P_i uptake in the presence of extravesicular P_i concentrations of 25–400 μmol/L K_2HPO_4. Finally, to examine whether the effect of dexamethasone was specific for Na-P_i cotransport, we also measured Na^+ gradient-dependent glucose and proline uptake by a method identical to that of P_i uptake except in the presence of 25 μmmol/L [^{3}H] D-glucose or 25 μmol/L [^{3}H] L-proline. A stop solution that consisted of 150 mmol/L NaCl, 0.25 mmol/L phloridzin, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50 was used in these experiments.

BBM phosphonoformic acid measurements. To determine whether the decrease in Na-P_i cotransport in dexamethasone-treated neonatal rabbits was associated with an alteration in the number of Na-P_i cotransport units, we measured P_i-protectable, Na^+-dependent equilibrium binding of PFA (Na-PFA) by a slight modification of the method previously described (20, 27). Briefly, freshly isolated BBM vesicles were either *1*) preloaded in a buffer consisting of 150 mmol/L NaCl, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50, and binding was initiated by mixing 10 μL of BBM with 40 μL of binding solution of same composition also containing 25 μmol/L [^{14}C]PFA (New England Nuclear, Boston, MA; sp act 30 mCi/mmol/L), or *2*) preloaded in a buffer of identical osmolality consisting of 100 mmol/L NaCl, 25 mmol/L Na_2HPO_4, 12.5 mmol/L choline chloride, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50, and binding was initiated by mixing 10 μL of BBM with 40 μL of binding solution of same composition also containing 25 μmol/L [^{14}C]PFA.

In preliminary studies we determined that maximal (equilibrium) binding of [^{14}C]PFA occurred at 30 min. Thus, we performed all the binding studies after 30 min incubation at 25°C. Binding was assayed after diluting and washing the unbound radioactivity by the addition of an ice-cold solution containing 150 mmol/L NaCl, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50. P_i-protectable Na-PFA binding is determined as the difference in PFA binding in *1*) the absence of P_i and *2*) presence of excess P_i, and it is expressed as pmol [^{14}C]PFA/30 min/mg BBM protein.

BBM lipid fluidity measurements. To determine whether the decrease in Na-P_i cotransport in dexamethasone-treated rabbits was associated with alterations in membrane lipid fluidity, BBM were isolated from control and dexamethasone-treated neonatal rabbit kidneys and fluidity was measured with fluorescence techniques. Steady-state fluorescence anisotropy of DPH (r_{DPH}) and TMA-DPH ($r_{TMA\text{-}DPH}$) were measured in a spectrofluorometer (SLM 4800C, Urbana, IL) equipped with excitation and emission polarizers as we have previously described (20, 21). Briefly, 0.1 mg BBM protein was resuspended in 150 mmol/L NaCl, 16 mmol/L HEPES, 10 mmol/L Tris, pH 7.50 buffer, and DPH or TMA-DPH were added from a stock solution to result in a probe/lipid ratio of 1:360. Excitation wavelength was 360 nm, and emission was viewed through a KV 399 nm filter. r_{DPH} and $r_{TMA\text{-}DPH}$ were determined by

$$r = \frac{I_{II} - I_I}{I_{II} + 2I_I}$$

where I_{II} and I_I represent the intensities of the parallel and perpendicular components of the emission, respectively. The parameter r_{DPH} or $r_{TMA\text{-}DPH}$ reflects the degree to which the rotation of DPH or TMA-DPH molecules embedded in BBM are hindered and provides an index that is inversely related to membrane lipid fluidity. Of note, the term membrane lipid fluidity is used here to denote the structural and dynamic properties that determine the relative motions and order of lipid molecules in the membrane.

BBM lipid composition determination. Total lipids in 1 mg BBM protein were extracted twice and evaporated under nitrogen as previously described (28). The sample was resuspended in chloroform and divided into aliquots for measurement of cholesterol, total phospholipids, and individual phospholipid content. The cholesterol sample was evaporated to residue, resuspended in hexane, and injected into a 530-μm 50% phenylmethyl silicone column (Hewlett-Packard, Palo Alto, CA) in a Hewlett-Packard model 5890 gas chromatograph with flame ionization detector run isothermally at 280°C. Coprostenol (Supelco, Bellefonte, PA) served as the internal standard. The areas were computed with a Hewlett Packard 3392A integrator, and the cholesterol content was expressed as nanomoles per milligram

BBM protein (21, 29). The sample for total phospholipid determination was dried to residue and resuspended in chloroform. The total phospholipid content was determined by measuring the phosphorus content as described by Ames and Dubin (30). Individual phospholipids were isolated by thin-layer chromatography. The sample was placed onto Kieselgel Silica Gel 60 precoated thin-layer chromatography plates (Merck, Darmstadt, Germany). The plates were developed in two dimensions. The first solvent was chloroform/methanol/acetic acid (65:25:10 vol/vol), and the second was chloroform/ethanol/88% formic acid (65:25:10 vol/vol). The chromatograms were allowed to dry and were then exposed to iodine. The individual phospholipids were well separated, and their location was compared with standards (Supelco, Bellefonte, PA). Areas of the silica gel containing phospholipids were scraped into acid-washed tubes, and the phospholipids were extracted from the silica gel (29). The silica gel was removed by centrifugation, and the phospholipids were separated into the chloroform phase. The sample was dried, and the phospholipid content was determined (30). Total phospholipid content was expressed as nanomoles per milligram BBM protein, and individual phospholipids were expressed as mol% of total phospholipid content.

Statistical analysis. All data are expressed as means ± SEM. Unpaired *t* test was used to compare results between control and dexamethasone-treated neonatal rabbits. Significance was accepted at $p < 0.05$.

RESULTS

Effect of dexamethasone on fractional excretion of phosphate. Dexamethasone administration for 4 consecutive d resulted in a significant increase in the fractional excretion of phosphate [0.4 ± 0.2 in control *versus* 13.7 ± 3.7% in dexamethasone-treated neonates ($p < 0.01$)].

Effect of dexamethasone on BBM enrichment and enzyme activity. BBM isolated from control and dexamethasone-treated neonates were equally enriched, 8- to 10-fold, as assessed by the activities of BBM-specific enzymes. Cross contamination with BLM was minimal and similar, as assessed by the activity of the BLM-specific enzyme Na^+-K^+-ATPase (Table 1). Dexamethasone administration resulted in significant increases in the activities of alkaline phosphatase (BBM bound) and Na^+-K^+-ATPase (BLM bound), whereas leucine aminopeptidase and γ-glutamyltransferase activities were not changed (Table 1).

Effect of dexamethasone on BBM transport activity. Dexamethasone treatment caused a significant decrease in BBM Na-P_i cotransport activity [50.8 ± 3.6 in control *versus* 29.2 ± 2.6 pmol $^{32}P_i$/10 s/mg BBM protein in dexamethasone-treated neonates ($p < 0.001$)] (Table 2). No significant difference was seen in Na-independent P_i transport (7.1 ± 1.6 in control *versus* 4.9 ± 0.8 pmol $^{32}P_i$/10 s/mg BBM protein in dexamethasone-treated neonates).

Table 1. *Effect of dexamethasone on membrane enzymes*

	Control	Dexamethasone	*p* value
Alkaline phosphatase			
Cortical homogenate	188.3 ± 12.3	310.3 ± 25.7	<0.001
BBM	1522.7 ± 59.9	2959.9 ± 243.9	<0.001
Enrichment	8.2 ± 0.3	9.6 ± 0.3	<0.005
Leucine aminopeptidase			
Cortical homogenate	66.5 ± 2.8	54.4 ± 5.4	NS
BBM	592.4 ± 98.3	497.5 ± 28.8	NS
Enrichment	8.9 ± 1.1	9.2 ± 0.4	NS
γ-Glutamyltransferase			
Cortical homogenate	316.7 ± 29.0	308.8 ± 26.4	NS
BBM	2615.5 ± 258.8	2632.3 ± 157.2	NS
Enrichment	8.3 ± 0.4	8.7 ± 0.3	NS
Na^+-K^+-ATPase			
Cortical homogenate	77.7 ± 16.2	145.9 ± 19.8	<0.025
BBM	80.0 ± 12.4	196.9 ± 23.1	<0.005
Enrichment	1.3 ± 0.3	1.5 ± 0.3	NS

Table 2. *Effect of dexamethasone on Na-P_i, Na-glucose, and Na-proline transport in BBM vesicles from neonatal rabbits*

	Control	Dexamethasone
Na-P_i (pmol ^{32}P/10 s/mg protein)	50.8 ± 3.6	29.2 ± 2.6*
Na-glucose (pmol ^3H glucose/10 s/mg protein)	31.4 ± 3.3	51.3 ± 5.1†
Na-proline (pmol ^3H proline/10 s/mg protein)	25.0 ± 3.2	41.2 ± 4.0†

* $p < 0.001$ *vs* control.
† $p < 0.05$ *vs* control.

BBM transport studies in the presence of 25–400 μmol/L extravesicular P_i demonstrated that in dexamethasone-treated neonates the decrease in Na-P_i cotransport was mediated by a decrease in the V_{max} [769 ± 21 in control *versus* 401 ± 30 pmol $^{32}P_i$/10 s/mg BBM protein in dexamethasone group ($p < 0.01$)]. No change was observed in the affinity (Km) for P_i [146 ± 9 in control neonates *versus* 142 ± 25 μmol/L P_i in dexamethasone-treated neonates (p = NS)]. These data are shown in Figure 1.

In contrast to the inhibition of Na-P_i cotransport, dexamethasone caused significant increases in Na-glucose [31.4 ± 3.3 in control neonates *versus* 51.3 ± 5.1 pmol ^3H glucose/10 s/mg BBM protein in dexamethasone-treated neonates ($p < 0.05$)] (Table 2) and Na-proline cotransport [25.0 ± 3.2 in control neonates *versus* 41.2 ± 4.0 pmol ^3H proline/10 s/mg BBM protein in dexamethasone-treated neonates ($p < 0.05$)] (Table 2).

Effect of dexamethasone on BBM P_i-protectable Na-PFA binding. Dexamethasone treatment caused no changes in total (results not shown) or P_i-protectable Na-PFA binding [7.4 ± 1.2 in control *versus* 7.7 ± 0.6 pmol [^{14}C]PFA/30 min/mg BBM protein in dexamethasone (p = NS)]. These results suggest that dexamethasone treatment does not cause an alteration in BBM Na-P_i cotransporter number (27).

Effect of dexamethasone on BBM fluidity. Dexamethasone treatment caused significant increases in the fluorescence anisotropy of DPH [0.214 ± 0.003 in control *versus* 0.222 ± 0.002 in dexamethasone group ($p < 0.05$)] and TMA-DPH [0.253 ± 0.001 in control *versus* 0.257 ± 0.001 in dexamethasone group ($p < 0.05$)]. These results suggest that dexamethasone treatment caused a decrease in BBM fluidity.

Effect of dexamethasone on BBM lipid composition. Dexamethasone treatment caused no change in BBM cholesterol or total phospholipid content. A significant decrease occurred in sphingomyelin, and a significant increase occurred in both phosphatidylcholine and phosphatidylinositol in BBM (Table 3).

DISCUSSION

The present study examined whether glucocorticoids are a potential factor to explain the maturational decrease in renal phosphate transport. The data demonstrate that dexamethasone-treated neonatal rabbits had a significant increase in fractional excretion of phosphate compared with control neonates. BBM vesicles from dexamethasone-treated neonates had a significantly lower rate of Na-P_i transport, which was due to a decrease in the V_{max} with no change in the Km of the transporter for P_i. This decrease in V_{max} was not mediated by a change in the number of transporters as assessed by P_i-protectable Na-PFA binding but was associated with an alteration in membrane fluidity and with changes in BBM phospholipid content.

Roberts and Pitts (31) first demonstrated that glucocorticoids inhibit the maximal tubular reabsorption of phosphate in normal and adrenalectomized dogs. Subsequent studies have shown that the phosphaturic effect of glucocorticoids was independent of

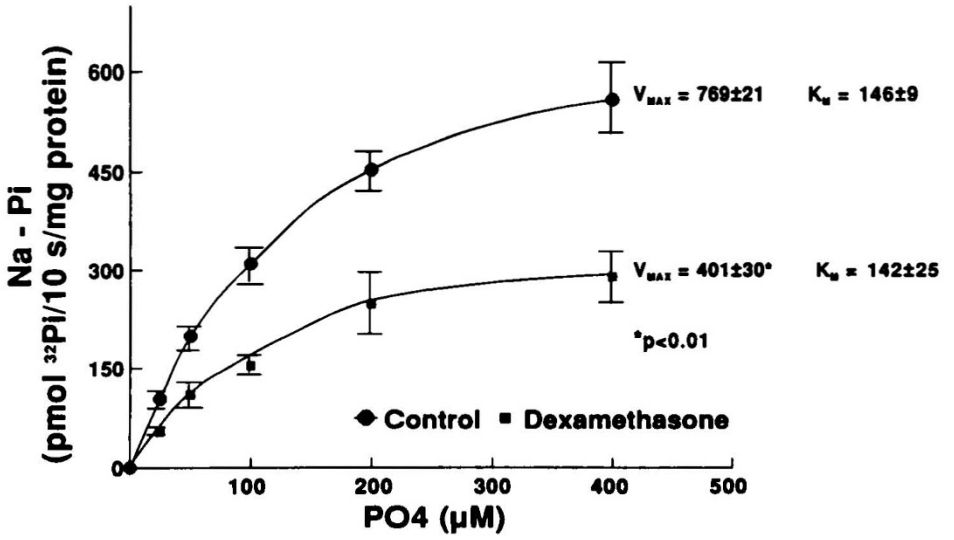

Fig. 1. Effect of dexamethasone on Na-P_i transport kinetics.

Table 3. *Effect of dexamethasone on BBM composition**

	Cholesterol	Total phospholipid	SPH	PC	PE	PS	PI
Control	349 ± 22	499 ± 19	25.3 ± 1.0	33.9 ± 1.1	26.0 ± 1.4	10.8 ± 0.8	3.9 ± 0.2
DEX	332 ± 14	499 ± 8	22.0 ± 0.7†	37.0 ± 1.0†	25.9 ± 0.3	10.6 ± 0.9	4.6 ± 0.2†

* Values are means ± SEM. Cholesterol and total phospholipid content are expressed as nanomoles per milligram BBM protein. Individual phospholipids are expressed as molar percentage of total phospholipid content. SPH, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; DEX, dexamethasone.

† $p < 0.05$ *vs* control.

parathyroid hormone (32–34) and the filtered load of phosphate (32, 34). The rate of Na-P_i uptake in renal BBM vesicles from adult rats who received glucocorticoids is slower than control rats (34, 35). The inhibitory effect of glucocorticoids on Na-dependent phosphate uptake was due to a decrease in V_{max} with no change in the Km for P_i (34). More recently, studies have demonstrated that glucocorticoids have a direct renal epithelial action *in vitro* to inhibit phosphate transport (36, 37). The direct inhibitory effect of glucocorticoids on Na-dependent P_i uptake in cultured chick renal cells was blocked by inhibitors of RNA and protein synthesis (36). The present study demonstrates that neonates have a similar response to glucocorticoids as do adult animals. Control neonates had virtually no phosphate in their urine, unlike the animals receiving dexamethasone. Renal BBM vesicles from dexamethasone-treated neonates had a lower rate of Na-dependent phosphate uptake, which was due to a lower V_{max} with no change in the Km for phosphate. The glucocorticoid-induced changes in membrane fluidity and lipid composition in our study may depend on RNA and protein synthesis *in vivo*. This possibility will require further investigation.

Previous studies have examined the effect of glucocorticoids on renal glucose transport. Na-dependent glucose transport in renal BBM vesicles from glucocorticoid-treated adult animals was no different than control adult animals (34, 35). Neonatal proximal tubules have a lower rate of glucose (3, 4) and proline (38) transport than adult proximal tubules. Although prenatal administration of glucocorticoids has been shown to accelerate the maturation of the renal apical Na^+/H^+ antiporter (2, 7), basolateral $Na(HCO_3)_3$ symporter (2), and basolateral Na^+-K^+-ATPase activity (1, 8–10), it had no effect on glucose transport (2, 7). In the present study, we measured Na-dependent glucose and proline transport as a control to determine whether there was a nonspecific inhibition in BBM transport in dexamethasone-treated neonates. Surprisingly, the rate of glucose and proline uptake were significantly higher in BBM vesicles from dexamethasone-treated neonates.

Previous studies have demonstrated that changes occur in the physical properties of the renal BBM with maturation (4, 39, 40). In both the rabbit (4) and rat (38, 40) an increase occurs in the fluorescence anisotropy of DPH (*i.e.* a decrease in membrane fluidity) in adult as compared with neonatal renal BBM. This change in membrane fluidity may play a role in maturational changes in membrane transport (39). In a previous study from our laboratory, we enriched the cholesterol content of BBM vesicles (20). An increase in cholesterol content of 12 and 24% resulted in a progressive decrease in BBM fluidity. This decrease in membrane fluidity was accompanied by a parallel decrease in Na^+-dependent P_i uptake. A decrease in membrane fluidity did not affect Na-glucose, Na-proline, and Na-H antiporter activity.

Evidence exists, however, that changes in membrane fluidity are not specific for phosphate transport. Other laboratories have found that increasing membrane fluidity by the addition of benzyl alcohol resulted in an increase in phosphate transport but a decrease in Na-glucose cotransport (41, 42). In addition, whereas butanol, hexanol, and heptanol all increase renal BBM fluidity, heptanol and hexanol had no effect and butanol decreased phosphate transport (43). Each alcohol lowered Na-proline transport. Thus, the glucocorticoid-induced decrease in membrane fluidity in our study may not be the cause for the decrease in phosphate transport.

In the present study we found that renal BBM from dexamethasone-treated neonates had a decrease in fluidity, a decrease in sphingomyelin, and an increase in phosphatidylcholine and phosphatidylinositol content. Membrane fluidity is determined by a number of factors, including protein-lipid interactions, cholesterol/phospholipid molar ratio, saturated/unsaturated acid molar ratio, sphingomyelin/phosphatidylcholine molar ratio, and glycolipid content. Although the decrease in sphingomyelin/phosphatidylcholine ratio observed after dexamethasone administration in our study is usually associated with an increase in membrane fluidity, this result is dependent on the fatty acid composition of the sphingomyelin and phosphatidylcholine (44).

A previous study has compared the lipid composition in intestinal BBM in suckling and weaned rats (45). Intestinal maturation was associated with a decrease in membrane fluidity accompanied by a decrease in sphingomyelin/phosphatidylcholine molar ratio. The maturational differences in membrane lipid fluidity were no longer observed in lipid extracts from these membranes, indicating that protein-lipid interactions, rather than lipid composition per se, mediated the maturational differences in membrane fluidity.

In the present study we found that administration of dexamethasone to neonatal rabbits resulted in a decrease in Na-dependent phosphate uptake in renal BBM vesicles. The dexamethasone-induced inhibition was due to a decrease in the V_{max} with no change in the Km for P_i. No change occurred in phosphate-protectable Na-PFA binding. Although PFA has been shown to bind to other anion cotransporters and thus may only be an approximation of the density of phosphate transporters (46), our results suggest that dexamethasone does not affect Na-phosphate transporter density and affinity. The inhibition in phosphate transport was accompanied by a reduction in BBM fluidity and alteration in individual phospholipid composition, with no change in either cholesterol or total phospholipid content. Conclusions about the role of glucocorticoids in renal maturation from studies using pharmacologic doses of dexamethasone must be made with caution. Similar changes in phosphate transport with glucocorticoids are observed in adult animals (but not glucose and proline transport), and thus the direct role of glucocorticoids in maturation of phosphate transport remains speculative. Whether physiologic changes in glucocorticoid concentration in neonatal animals are associated with changes in phosphate transport and BBM composition and fluidity cannot be and were not directly addressed. However, our data suggest that glucocorticoids may play a role in the postnatal decrease in renal phosphate transport.

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