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Vitamin D metabolites stimulate phosphatidylcholine transfer to renal brush-border membranes

Brenda R.C. Kurnik *, Margaret Huskey, David Hagerty and Keith A. Hruska

Renal Division, Department of Medicine, Washington University and Jewish Hospital, St. Louis, MO 63110 (U.S.A.)

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The phosphatidylcholine content of both the intestinal and renal brush-border membranes and ion transport are affected by 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). To investigate the mechanism of this effect, liposomes were prepared containing self-quenching concentrations of fluorescent phospholipid derivatives. When these liposomes were incubated with rat renal brush-border membrane vesicles, an immediate increase in the relative fluorescence of *N*-4-nitrobenz-2-oxa-1,3-diazole phosphatidylcholine (NBD-PC) was detected, indicating transfer of NBD-PC into a non-quenched membrane. Addition of $1,25(OH)_2D_3$ to the liposomes produced a dose-dependent stimulation of NBD-PC transfer to the acceptor brush-border membrane vesicles. Peripheral fluorescence was visible when the brush-border membrane vesicles were viewed with a fluorescent microscope. Using brush-border membrane vesicles from kidneys of vitamin D-deficient animals, quantitation of lipid transfer revealed a $1,25(OH)_2D_3$ (10^{-7} M) stimulation of NBD-PC transfer from 1.38 ± 0.27 to 2.07 ± 0.26 μg/h, and of PC transfer, assessed by vesicle phosphatidylcholine content, from 49.7 ± 12 to 57.3 ± 12 μg/mg protein per h ($P < 0.05$). There was no significant transfer of *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine (*N*-Rh-PE). In the absence of hormone, the amount of NBD-PC transferred to brush-border membrane vesicles prepared from normal rats was significantly greater than that transferred to brush-border membrane vesicles prepared from vitamin D-deficient animals (2.12 ± 0.02 vs. 1.39 ± 0.27 μg of NBD-PC/h, $P < 0.05$). Both physiologic and pharmacologic concentrations of $1,25(OH)_2D_3$ stimulated NBD-PC transfer with maximum response at 10^{-14} M (2.98 ± 0.15 μg/h). 24,25-Dihydroxycholecalciferol and 25-hydroxycholecalciferol ($25(OH)D_3$) also stimulated transfer, although dose-response curves were less effective than for $1,25(OH)_2D_3$. Cortisol and vitamin D-3 did not stimulate transfer. $1,25(OH)_2D_3$ did not stimulate NBD-PC transfer between liposome populations.

* To whom correspondence should be addressed at: Jewish Hospital of St. Louis, Renal Division, 216 South Kingshighway, St. Louis, MO 63110, U.S.A.

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; $25(OH)D_3$, 25-hydroxycholecalciferol; $24,25(OH)_2D_3$, 24,25-dihydroxycholecalciferol; NBD-PC, 1-acyl-2-(*N*-4-nitrobenz-2-oxa-1,3-diazole)-aminocaproylphosphatidylcholine; PC, phosphatidylcholine; DOPC, dioleoylphosphatidylcholine; *N*-Rh-PE, *N*-(lissamine rhodamine B sulfonyl)-dioleoylphosphatidylethanolamine; Mes, 4-morpholineethanesulfonic acid.

Introduction

Current evidence suggests that the mechanisms of action of several hormones may be mediated through alterations in membrane lipid composition [1–4]. Both the content and the degree of fatty acid unsaturation of phosphatidylcholine are

increased in duodenal [6] and renal proximal tubular brush-border membranes [5] after (1,25-$(OH)_2D_3$ administration. In the intestine, these lipid changes were shown to be important in stimulating calcium and possibly phosphate transport [5,7]. When the relationship between phosphate transport and temperature was analyzed in renal brush-border membrane vesicles, construction of Arrhenius plots showed that $1,25(OH)_2D_3$ induced changes in both the slopes and transition temperatures of the plots [8]. These data supported a lipid-mediated mechanism of action. Because of a paucity of information regarding normal membrane lipid synthesis and processing, and only recent recognition that hormonal effects may be mediated through direct changes in membrane lipids, little work has been performed to determine the mechanism by which these lipid changes are produced. The following study was performed to determine if $1,25(OH)_2D_3$ could directly increase membrane phosphatidylcholine content by stimulating phospholipid transfer between membranes. It was found that $1,25(OH)_2D_3$, $25(OH)D_3$ and $24,25(OH)_2D_3$ specifically facilitate the transfer of phosphatidylcholine analogs from one membrane, a liposome, to the renal brush-border membrane. Stimulation of lipid transfer may represent a general and direct method of membrane phospholipid modification induced by hormones.

Experimental procedures

Animal model. A rat model of partial vitamin D deficiency, devoid of the usual rachitic complications of hyperparathyroidism and changes in serum calcium and phosphate levels, has been developed by feeding weanling Sprague-Dawley rats a diet deficient in vitamin D but high in calcium (1.8%) and phosphorus (1.2%) for 5–6 weeks. These animals have normal levels of serum calcium, phosphorus and parathyroid hormone, undetectable serum 25(OH)D and reduced $1,25(OH)_2D$ levels [9]. Brush-border membrane vesicles were also prepared from weight-matched normal rats.

Preparation of brush-border membrane vesicles. The rats were anesthetized with ether and blood was drawn from the inferior vena cava prior to bilateral nephrectomy. The kidneys were immediately placed on ice, and brush-border membrane vesicles were prepared by a technique of divalent cation precipitation and differential centrifugation [9]. The final vesicle preparation was suspended in 150 mM KCl/2 mM Mes-Tris (pH 7.5) to achieve a protein concentration of between 1.0 and 2.0 mg/ml.

This preparation has been previously characterized [9] as being enriched in alkaline phosphatase activity, a marker of the brush-border membrane and decreased in enrichment of sodium potassium ATPase activity, a marker of the basolateral membrane.

Preparation and characterization of liposomes. Small unilamellar liposomes composed of dioleoylphosphatidylcholine (DOPC), with or without phospholipid derivatives which contained fluorescent groups in either the No. 2 fatty acid (NBD-PC) or head-group (*N*-Rh-PE) position (Avanti Polar Lipids, Birmingham, AL) were prepared as previously described by Barenholz et al. [10]. The concentrations of fluorescent phospholipids in the liposomes were chosen so as to 'self-quench', that is, the liposomes photographed alone did not fluoresce, and the measurable fluorescence was minimal, 0.015 for NBD-PC and 0.04 for *N*-Rh-PE. Self-quenching concentrations were determined to be 30 mol% for NBD-PC and 20 mol% for *N*-Rh-PE.

The liposomes were prepared as follows: phospholipids (500 μg) with or without a hormonal addition were dried under a stream of nitrogen and lyophilized for 2 h to remove all trace solvent. They were reconstituted in 400 μl of 150 mM KCl/2 mM Mes-Tris (pH 7.5) and sonicated to clarity under nitrogen in a bath sonicator (Laboratory Supplies, Hicksville, NY). The volume was increased to 5 ml and the liposomes ultracentrifuged at $159000 \times g$ for 90 min. The supernatants contained only small unilamellar liposomes, 25–75 nm in diameter as characterized by negative staining electron microscopy and column chromatography. To further characterize the liposomes prepared by ultracentrifugation, they were subjected to gel chromatography on a Sepharose 4B column (2.5×50 cm) at 4°C pretreated with 50 mg of egg phosphatidylcholine. The liposome preparation was applied to the column and the effluent collected in 5-cm^3 fractions at a rate of 0.5 cm^3/min. The absorbance at 300 nm was measured on a

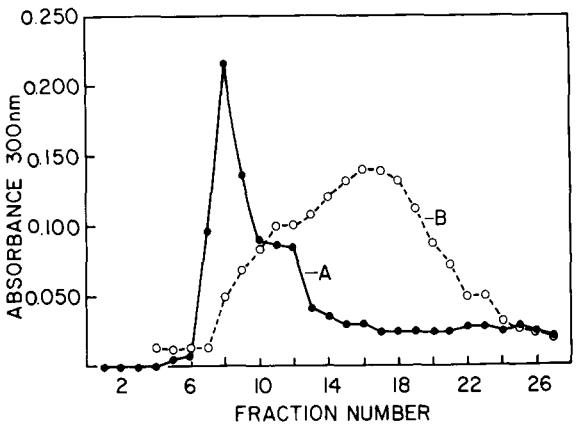

Fig. 1. Elution profile of liposomes before and after ultracentrifugation. Liposomes composed of DOPC were prepared and passed through a Sepharose 4B column as described in 'Experimental procedures'. Curve A represents the elution profile of the preparation before ultracentrifugation and curve B the elution profile of the supernatant after ultracentrifugation (159000 × *g* for 90 min).

LKB spectrophotometer. Fig. 1 shows the results of column chromatography using the entire liposome preparation either before (curve A) or after (curve B) ultracentrifugation. Prior to ultracentrifugation, the liposome population is heterogeneous containing large unilamellar or multilamellar structures as shown in Fig. 2A. The multilamellar and large liposomes elute early upon column fractionation. Following ultracentrifugation, the specimen was enriched for a population of smaller unilamellar liposomes (Fig. 2B) which elute in later fractions (Fig. 1, curve B). The supernatant which contained fractions 14 and above were obtained and used for the experiments described in this report. The negative staining electron microscopy was performed courtesy of the Diabetes Research Training Center, Washington University School of Medicine (AM 20579). The electron micrographs and chromatographic elution pat-

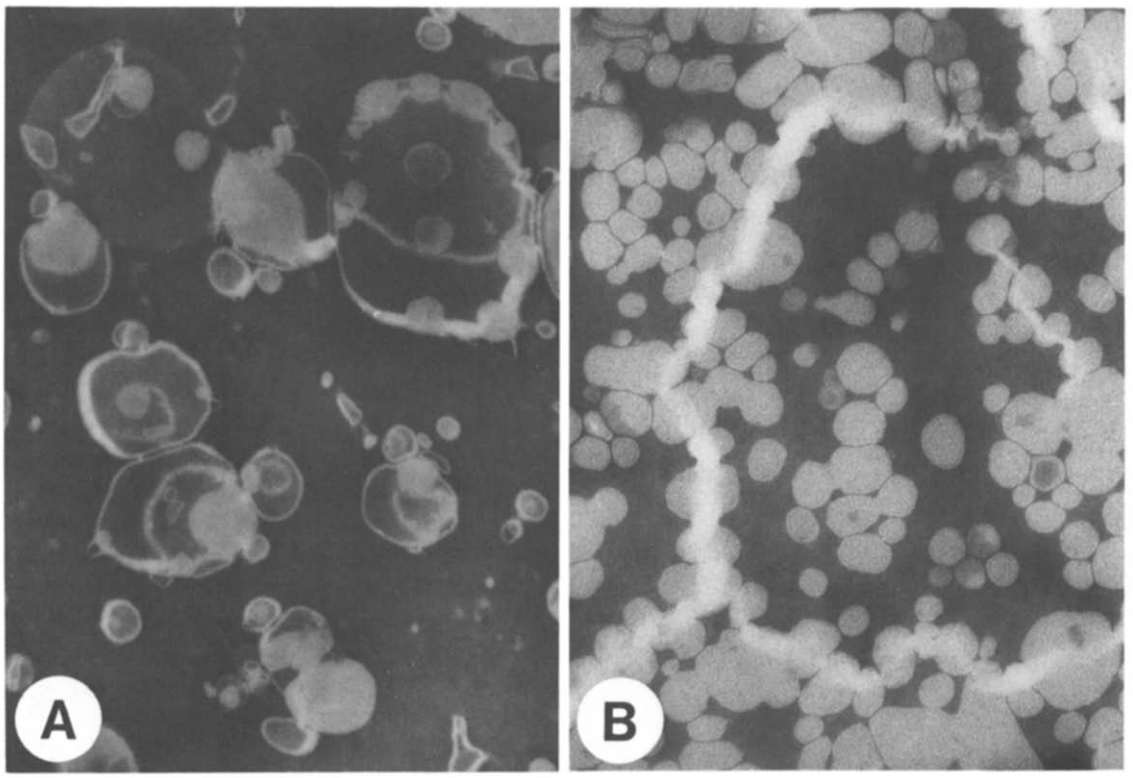

Fig. 2. Negative staining electron micrographs of liposomes. Liposomes composed of DOPC were prepared as described under 'Experimental procedures' and negative staining electron microscopy was performed. Panel A shows a population of multilamellar, large liposomes (magnification ×18000; 1 μm = 0.72 in.) prior to ultracentrifugation. Panel B shows a population of small unilamellar liposomes following ultracentrifugation (magnification ×45000; 1 μm = 1.8 in.).

terns of the liposomes were the same in the presence or absence of hormone or phospholipid additions.

Spectrofluorometry. Relative fluorescence was measured using an SLM 4880 spectrofluorometer (Urbana IL). NBD-PC was excited at 467 nm, and emission at 528 nm was recorded. For *N*-Rh-PE, an excitation of 553 nm was used and emission at 583 nm was determined. The change in fluorescent intensity with time was continuously monitored at the rate of one measurement every second, relayed to a computer and recorded.

Fluorescent microscopy. Fluorescent microscopy was performed with the use of a Nikon upright fluorescent microscope equipped with barrier filters that prevented any crossover between the nitrobenzoxadiazole and rhodamine fluorescence.

Incubation of vesicles with liposomes. The liposomes containing 'self-quenching' concentrations of fluorescent phospholipids with or without hormones were incubated with brush-border membrane vesicles to give final concentrations of 0.5–1 mg/ml of vesicle protein and 50 μg/ml of liposome lipid. The hormones studied included $1,25(OH)_2D_3$ at both pharmacologic and physiologic concentrations, $25(OH)D_3$, $24,25(OH)_2D_3$, cortisol and vitamin D-3 at the concentrations specified. A decrease in the fluorescent phospholipid concentration was measurable as an increase in fluorescence. This could occur through either a direct transfer of fluorescent phospholipid molecules to vesicle membranes or fusion of liposomes with the brush-border membrane vesicles. NBD-PC has been previously shown to transfer spontaneously between membranes, while *N*-Rh-PE does not [11]. Therefore, the appearance of both NBD-PC and *N*-Rh-PE in a vesicle membrane would represent either a fusion event or non-specific adhesion. However, the selective appearance of NBD-PC would support phospholipid exchange or transfer. The transfer from liposome to vesicle of either NBD-PC or *N*-Rh-PE was monitored by measuring the increase in relative fluorescence at 0°C for 5–15 min at the appropriate wavelengths.

Quantitation of lipids. In order to measure the amount of fluorescent phospholipid transferred, vesicles were incubated with liposomes composed of 30 mol% NBD-PC, 2 mol% *N*-Rh-PE, 68 mol% DOPC with or without a steroid hormone for 60 min at 0°C. The vesicles were then pelleted by centrifugation ($35\,000 \times g$), the supernatant (free liposomes) was discarded and the pellet was washed two more times. The pellets containing vesicle lipid were extracted as described by Bligh and Dyer [12] using chloroform/methanol/0.1 M HCl (1 : 2 : 8). Lipid extracts were analyzed by chromatography on silica gel 60 thin-layer plates (E. Merk, Darmstadt, F.R.G.) in chloroform/methanol/28% ammonium hydroxide (65 : 35 : 5, v/v) along with standards including *N*-Rh-PE and NBD-PC. The spots containing *N*-Rh-PE and NBD-PC were scraped and re-extracted as by Bligh and Dyer [12] with chloroform/methanol/water (1 : 2 : 0.8, v/v). The amount of fluorescent lipid retrieved was quantitated by comparing the amount of fluorescence in a sample to the fluorescence of a standard curve generated using known concentrations of either *N*-Rh-PE or NBD-PC. Non-fluorescent phospholipid was quantitated by P_i assay by the method of Ames [13].

Analytic methods. The Student's *t*-test for non-paired data was used for comparisons between groups when the brush-border membrane vesicles were derived from different animals. For comparison of results, using brush-border membrane vesicle preparations from the same animals, a paired Student's *t*-test was used.

Materials. Fluorescent phospholipids were obtained from Avanti Polar Lipids (Birmingham, AL); they were periodically monitored for purity by thin-layer chromatography and re-purified as necessary. Vitamin D metabolites were a gift from Hoffman LaRoche (Nutley, NJ). Other chemicals were of the highest purity available from commercial sources. All solutions were filtered through a 0.45 μm Millipore filter on the day of the experiment.

Results

Time-course of transfer of fluorescent phospholipids to brush-border membrane vesicles

When liposomes composed of 30 mol% NBD-PC were incubated with brush-border membrane vesicles prepared from kidneys of partially vitamin D-deficient rats, an increase in relative fluorescence was immediately detectable, reaching a

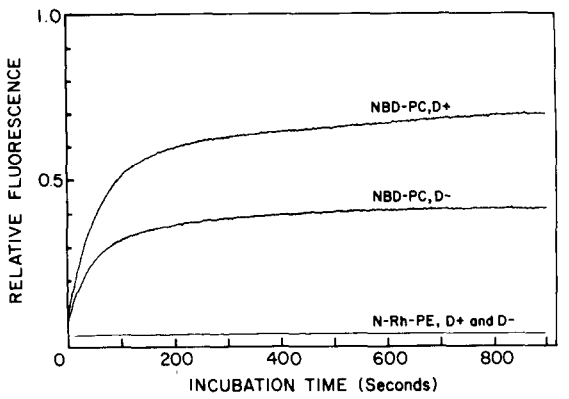

Fig. 3. Effect of calcitriol on the transfer of fluorescent phospholipids from liposomes to brush-border membrane vesicles from vitamin D-deficient rats. Liposomes containing self-quenching concentrations of either NBD-PC (30 mol%) or *N*-Rh-PE (20 mol%) in addition to DOPC were incubated with brush-border membrane vesicles for 15 min at 0°C. The increase in fluorescence was monitored at the appropriate wavelenghts (NBD-PC, excitation 467 nm, emission 528 nm; *N*-Rh-PE, excitation 553 nm, emission 583 nm). This figure represents one of six experiments with similar results.

level of 0.31 fluorescent units at 100 s and 0.41 at 15 min. Liposomes containing 10^{-7} M 1,25-$(OH)_2D_3$ (a pharmacologic dose) stimulated the initial rate and amount of NBD-PC fluorescence to 0.52 at 100 s and 0.70 at 15 min (Fig. 3). When

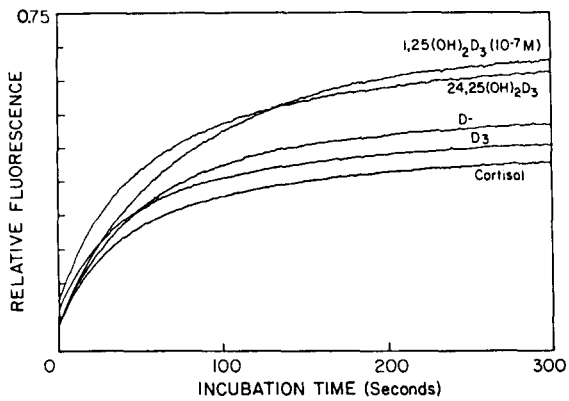

Fig. 4. Effects of several hormones on the transfer of NBD-PC from liposomes to brush-border membrane vesicles from normal rats. Liposomes composed of 30 mol% NBD-PC and 70 mol% DOPC with or without hormonal additions (10^{-7} M) were incubated with brush-border membrane vesicles derived from normal rats. The relative fluorescence was monitored for 5 min at 0°C, excitation 467 nm and emission 528 nm. This is a representative example of four experiments.

liposomes containing 20 mol% of *N*-Rh-PE were incubated with brush-border membrane vesicles, there was no detectable fluorescence in the presence or absence of $1,25(OH)_2D_3$.

Liposomes composed of 30 mol% NBD-PC were also incubated with brush-border membrane vesicles prepared from normal rats containing the same amount of membrane protein in the brush-border membrane vesicles used in Fig. 3. Fig. 4 demonstrates that both $1,25(OH)_2D_3$ and 24,25-$(OH)_2D_3$ (10^{-7} M) stimulated the rate of fluorescent NBD-PC transfer. Vitamin D-3 (10^{-7} M) did not stimulate phospholipid transfer, and cortisol (10^{-7} M) suppressed transfer.

Transfer of NBD-PC from liposomes to brush-border membrane vesicles isolated from kidneys of vitamin D-deficient animals (0.31 at 100 s, Fig. 3) was less than to brush-border membrane vesicles from replete animals (0.40 at 100 s, Fig. 4). This data is further confirmed below in the section on quantitation of lipid transferred. Incorporation of $1,25(OH)_2D_3$ (10^{-7} M) into the quenched liposomes increased transfer of NBD-PC by 68% at 100 s to brush-border membrane vesicles of kidneys from deficient animals (Fig. 3) and by 23% to brush-border membrane vesicles from normal animals (Fig. 4).

When $1,25(OH)_2D_3$ was not incorporated into the quenched fluorescent liposomes but merely added to the reaction mixture of liposomes and brush-border membrane vesicles as a separate addition, the stimulation of NBD-PC transfer to the brush-border membrane vesicles by $1,25(OH)_2D_3$ was still present but somewhat less. The stimulation was $8.3 \pm 0.5\%$, significantly greater than transfer of NBD-PC to brush-border membrane vesicles in the absence of $1,25(OH)_2D_3$.

To determine the effect of physiological concentrations of $1,25(OH)_2D_3$ on lipid transfer, a dose-response curve was performed. Fig. 5 demonstrates that $1,25(OH)_2D_3$ increased transfer at concentrations ranging from 10^{-7} to 10^{-17} M. The peak response was present at 10^{-14} M, and the effect was undetectable by 10^{-18} M. Thus, the observations depicted in Figs. 3 and 4 with pharmacologic doses of $1,25(OH)_2D_3$ were present at physiologic doses. Dose-response curves for $24,25(OH)D_3$ revealed peak effects at 10^{-7} M and disappearance of effect at 10^{-13} M.

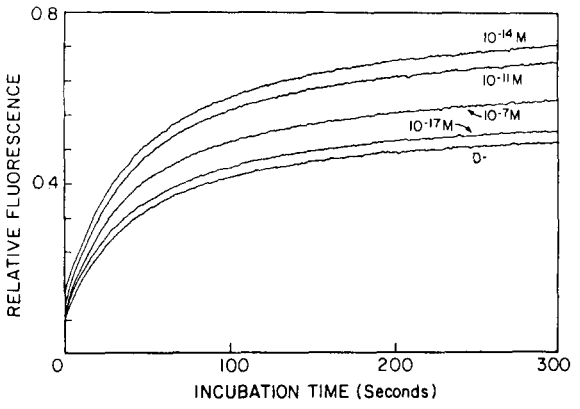

Fig. 5. Dose response of the effect of calcitriol on NBD-PC transfer. The effects of 10^{-7}, 10^{-11}, 10^{-14} and 10^{-17} M calcitriol on the transfer of NBD-PC from liposomes to brush-border membrane vesicles prepared from normal rats is demonstrated. Liposomes were composed of 30 mol% NBD-PC and 70 mol% DOPC with or without (D^-) hormonal additions. The relative fluorescence was monitored for 5 min at 0°C, excitation 467 nm and emission 528 nm.

Quantitation of lipid transferred

Table I shows the results of incubating liposomes composed of 30 mol% NBD-PC, 2 mol% *N*-Rh-PE and 68 mol% DOPC with brush-border membrane vesicles from partially vitamin D-deficient rats and quantitating the vesicle lipids. After 1 h at 0°C, $1,25(OH)_2D_3$ (10^{-7} M) stimulated the transfer of NBD-PC from 1.39 ± 0.27 to 2.07 ± 0.26 μg ($P < 0.05$). There was minimal transfer of *N*-Rh-PE in the presence or absence of hormone. The vesicle phosphatidylcholine content was also increased by $1,25(OH)_2D_3$ from 49.7 ± 12 to 57.3 ± 12 μg/mg protein (paired *t*-test, $P < 0.05$).

TABLE I

QUANTITATION OF TRANSFER OF NBD-PC, DOPC AND *N*-Rh-PE FROM LIPOSOMES TO BRUSH-BORDER MEMBRANE VESICLES PREPARED FROM VITAMIN D-DEFICIENT RATS

Liposomes composed of either 30 mol% NBD-PC, 2 mol% *N*-Rh-PE and 68 mol% of DOPC or DOPC with or without hormone were incubated with brush-border membrane vesicles from vitamin D-deficient rats at 0°C for 1 h. The vesicle phospholipids were extracted and quantitated as described under 'Experimental procedures'.

	D^-	$1,25(OH)_2D_3$ (10^{-7} M)
NBD-PC, μg/h ($n = 6$)	1.39 ± 0.27	2.07 ± 0.26 *
Vesicle PC content μg/mg protein per h ($n = 5$)	49.7 ± 12	57.3 ± 12 *
N-Rh-PE, ng/h ($n = 3$)	5 ± 3	4 ± 4

* $D^+ > D^-$, $P < 0.05$, paired *t*-test.

TABLE II

QUANTITATION OF TRANSFER OF NBD-PC FROM LIPOSOMES TO RENAL BRUSH-BORDER MEMBRANE VESICLES PREPARED FROM NORMAL RATS

Liposomes composed of 30 mol% NBD-PC, 70 mol% DOPC with or without hormones additions were incubated with brush-border membrane vesicles from normal rats at 0°C for 1 h. The vesicle phospholipids were extracted and quantitated as described under 'Experimental procedures'.

	n	NBD-PC (μg/h)
D^-	12	2.12 ± 0.02
$1,25(OH)_2D_3$ (10^{-7} M)	7	2.60 ± 0.23 *
$24,25(OH)_2D_3$ (10^{-7} M)	6	2.64 ± 0.24 *
$25(OH)D_3$ (10^{-7} M)	4	2.63 ± 0.14 *
D-3 (10^{-7} M)	7	2.27 ± 0.19
Cortisol (10^{-7} M)	3	1.64 ± 0.08 **

* $D^+ > D^-$, $P < 0.05$, paired *t*-test.

** Cortisol < D^-, $P < 0.01$, paired *t*-test.

TABLE III

QUANTITATION OF TRANSFER OF NBD-PC FROM LIPOSOMES TO RENAL BRUSH-BORDER MEMBRANE VESICLES PREPARED FROM NORMAL RATS

Liposomes composed of 30 mol% NBD-PC, 70 mol% DOPC and calcitriol, final concentrations as specified, were incubated with brush-border membrane vesicles from normal rats at 0°C for 1 h. The vesicle phospholipids were extracted and quantitated as described under 'Experimental procedures'.

	n	NBD-PC (μg/h)
D^-	12	2.12 ± 0.02
$1,25(OH)_2D_3$		
10^{-7} M	7	2.60 ± 0.22 *
10^{-11} M	4	2.63 ± 0.06 *
10^{-14} M	3	2.98 ± 0.15 *
10^{-17} M	4	2.44 ± 0.17 *
10^{-18} M	3	2.13 ± 0.20

* $D^+ > D^-$, $P < 0.01$.

When liposomes containing $1,25(OH)_2D_3$ were added to brush-border membrane vesicles prepared from normal rats, the NBD-PC content increased from 2.12 ± 0.02 to 2.60 ± 0.23 μg (10^{-7} M) ($P < 0.02$, $D^+ > D^-$). The transfer of NBD-PC to brush-border membrane vesicles derived from normal rats was significantly greater than to brush-border membrane vesicles derived from vitamin D-deficient animals ($P < 0.05$) in the absence of hormonal additions. The transfer of NBD-PC was also stimulated in the presence of 10^{-7} M $24,25(OH)_2D_3$ to 2.64 ± 0.23 μg ($P < 0.05$), and 10^{-7} M $25(OH)D_3$ to 2.63 ± 0.13 μg. Vitamin D_3, did not increase the transfer of NBD-PC. Cortisol significantly suppressed NBD-PC transfer to 1.64 ± 0.08 μg ($P < 0.001$) (Table II). A dose response was also performed to quantitate $1,25(OH)_2D_3$-stimulated transfer of NBD-PC. $1,25(OH)_2D_3$ stimulated transfer at concentrations ranging from pharmacologic 10^{-7} M to 10^{-17} M. The peak effect occurred at 10^{-14} M ($P < 0.01$) as seen in Table III and Fig. 5.

Microscopy

Liposomes alone containing 30 mol% NBD-PC or 20 mol% *N*-Rh-PE were self-quenching and were not visible when viewed under the fluorescent microscope. Vesicles alone had no detectable autofluorescence but could be visualized under the light microscope as seen in Fig. 6, panel A.

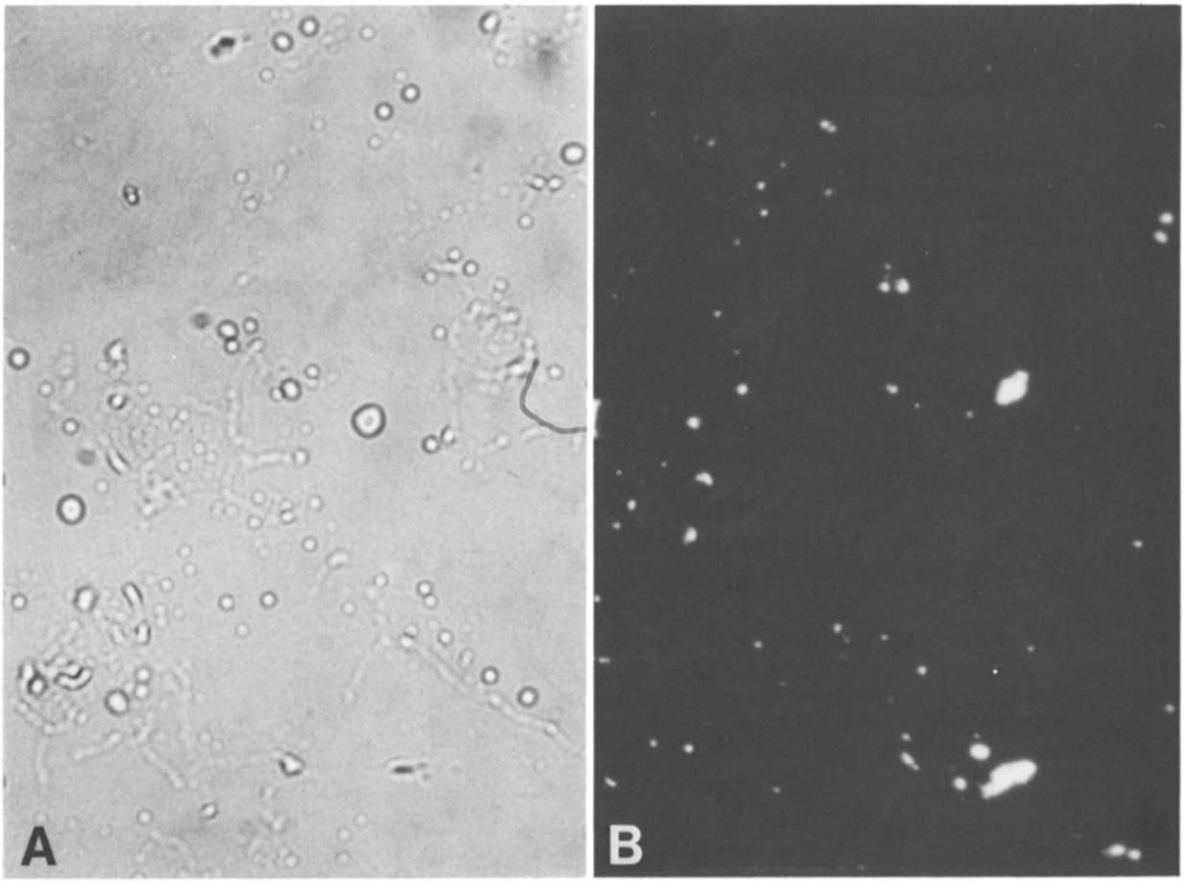

Fig. 6. Light and fluorescence micrographs of brush-border membrane vesicles. Panel A shows brush-border membrane vesicles photographed with a light microscope, magnification ×360. Panel B shows fluorescent brush-border membrane vesicles following a 5 min incubation at 0°C with liposomes containing 30 mol% NBD-PC, 2 mol% *N*-Rh-PE and 68 mol% DOPC (magnification ×360). Fluorescent excitation and emission were adjusted by barriers to the range in the legend of Fig. 3. Neither vesicles nor liposomes alone were detectably fluorescent. The field in panel B is different from that in panel A.

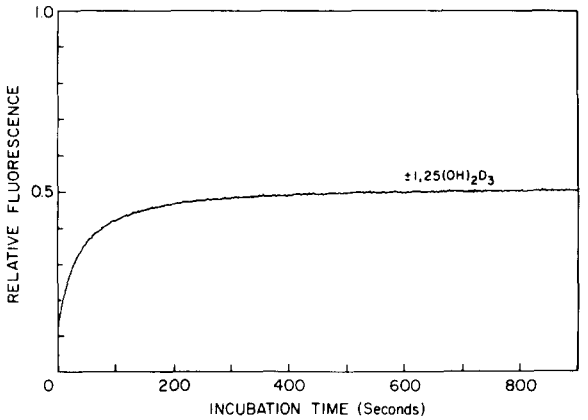

Fig. 7. Effect of calcitriol on the transfer of NBD-PC to non-fluorescent liposomes. Liposomes composed of 30 mol% NBD-PC and 70 mol% DOPC with or without calcitriol were incubated with liposomes composed of only DOPC at 0°C for 15 min. The relative fluorescence was monitored as described in the legend of Fig. 3. Several doses of $1,25(OH)_2D_3$ were used in these experiments: 10^{-7}, 10^{-11} and 10^{-14} M. None affected NBD-PC transfer from liposomes to liposome.

Following a 5-min incubation of NBD-PC containing liposomes with brush-border membrane vesicles, peripheral fluorescence was visible as shown in Fig. 6, panel B.

Transfer of fluorescent lipid between liposome populations

In order to determine whether the $1,25(OH)_2D_3$-induced stimulation of NBD-PC transfer was dependent on the nature of the accepting membrane, liposomes containing 30 mol% NBD-PC and 70 mol% DOPC with or without hormone were incubated with a second population of liposomes containing only DOPC. The initial relative fluorescence of the self-quenched NBD-PC liposomes was only 0.15 and the DOPC liposomes had no autofluorescence. Fig. 7 shows that NBD-PC did spontaneously transfer to the DOPC liposomes resulting in measurable fluorescence; however, it was the same in the presence or absence of $1,25(OH)_2D_3$.

Discussion

The present studies demonstrate transfer of fluorescent phosphatidylcholine analogues from quenched liposomes to brush-border membrane vesicles. Transfer rates of phospholipid were less to brush-border membrane vesicles from vitamin D-deficient animals than to vesicles from kidneys of normal animals. Thus, the present studies suggest that the increase in the phosphatidylcholine content of both the renal [5] and duodenal [6] brush-border membranes previously reported following $1,25(OH)_2D_3$ treatment may be partially mediated through a facilitation of the transfer of phosphatidylcholine to the brush-border membrane.

Membrane phospholipid biosynthesis occurs primarily in the endoplasmic reticulum with possible further modification in the Golgi apparatus. The lipids are then incorporated into the plasma membrane, either by direct transfer via phospholipid-exchange transfer proteins or by fusion of intracellular lipidic particles with the plasma membrane. The data presented herein suggest that $1,25(OH)_2D_3$ stimulates phospholipid transfer rather than fusion, since only the NBD-PC and not the *N*-Rh-PE of mixed liposomes was transferred. Because the property of spontaneous transfer of the fluorescent phospholipid derivatives is not physiologic, experiments were also performed using liposomes composed only of DOPC with or without $1,25(OH)_2D_3$. The results demonstrated that the stimulation of phospholipid transfer by $1,25(OH)_2D_3$ was not unique to fluorescent phosphatidylcholine. An increase in vesicle total phosphatidylcholine content occurred in the presence of $1,25(OH)_2D_3$ and the liposomes.

The transfer of NBD-PC was stimulated by $1,25(OH)_2D_3$ when the brush-border membrane vesicles were prepared from either partially vitamin D-deficient or normal rats. In the absence of $1,25(OH)_2D_3$, NBD-PC transfer was greater to brush-border membrane vesicles derived from normal rat kidneys. Since NBD-PC transfer between liposome populations was not increased by $1,25(OH)_2D_3$, its effect was most likely exerted on movement of PC into the acceptor membrane rather than on release of PC from the donor liposome. However, the decreased effectiveness of $1,25(OH)_2D_3$, when added directly to the suspension of brush-border membrane vesicles and liposomes rather than sonicated with the liposomes, is hard to understand.

Either the lipid composition or protein content of the brush-border membrane appears to be an important component of the process of lipid transfer and that differences in critical variables exist between brush-border membrane vesicles derived from partially vitamin D-deficient and normal animals. One possibility would be that a protein, possibly a phosphatidylcholine-transfer protein, was stimulated by $1,25(OH)_2D_3$. The differences in the stimulation of PC transfer to the two types of brush-border membrane vesicle could be explained by alterations in this protein. Alternatively, PC transfer could be dependent on the differences in membrane lipid composition between normal and vitamin D-deficient animals. Membrane lipid composition has been shown to play an important role in modulating both cholesterol- and protein-mediated phospholipid exchange between mycoplasma gallisepticum cells and liposomes [14]. Perhaps, brush-border membrane vesicles derived from normal rats accept the phosphatidylcholine more easily than brush-border membrane vesicles derived from vitamin D-deficient animals.

Both $24,25(OH)_2D_3$ and $25(OH)D_3$ in addition to $1,25(OH)_2D_3$ stimulated the transfer of NBD-PC to brush-border membrane vesicles. Vitamin D_3 did not stimulate transfer. This data suggests a specificity for a metabolite hydroxylated in the 25-position to produce this effect. Further studies will be required to characterize specific structural requirements and elucidate the mechanism of this effect. The dose response of $1,25(OH)_2D_3$ demonstrates that the maximum effect occurs at concentrations in the physiologic range. Cortisol significantly suppressed NBD-PC transfer. If the effect of $1,25(OH)_2D_3$ on phosphate transport is due to a liponomic mechanism of control [8] and varies with membrane phosphatidylcholine content, then suppression by cortisol of NBD-PC transfer may relate to the well-established phosphaturic effect of glucocorticoids. Further studies will be needed to evaluate the direct effects of alterations of membrane lipids on transport processes.

The liposome in these experiments could represent the equivalent of any intracellular membrane known to be involved in lipid synthesis, possibilities include the endoplasmic reticulum, Golgi membrane, or intracellular lipidic particles. Recent evidence suggests that $1,25(OH)_2D_3$ incorporated into a liposome may increase its calcium permeability and possibly its thermodynamic membrane stability, possibly modifying it to allow phosphatidylcholine transfer [15]. The studies presented here demonstrate that a steroid hormone facilitates specific lipid transfer, and this may be a general mechanism of action for other hormones thought to directly modify membrane lipid composition.

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