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Mechanism of stimulation of renal phosphate transport by 1,25-dihydroxycholecalciferol

Brenda R.C. Kurnik [a,b,*] and Keith A. Hruska [a,b]

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

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Vitamin D has been shown to stimulate renal phosphate transport and to alter membrane phospholipid composition. The present studies examine the possibility that the effects of $1,25(OH)_2D_3$ on phosphate transport are related to its effects on membrane lipids. Arrhenius plots, which relate maximum rates of sodium dependent phosphate uptake into brush-border membrane vesicles to temperature were constructed. Phosphate transport was studied using brush-border membrane vesicles from normal, vitamin D-deficient, and physiologically replete (15 pmol/100 g body weight per 24 h) rats. These plots were triphasic with characteristic, lipid-dependent, slopes (M_1, M_2, M_3) representing activation energies and transition temperatures (T_1, T_2). Physiologic $1,25(OH)_2D_3$ repletion normalized these plots by stimulating phosphate transport at all temperatures, increasing T_2 from 18 ± 0.7 to 23.5 ± 0.9°C and decreasing M_2 and M_3 from -5.8 ± 0.2 and -10.2 ± 0.4 to -4.5 ± 0.4 and -7.7 ± 0.3, respectively. Pharmacologic (1.2 nmol/100 g per 3 h) $1,25(OH)_2D_3$ treatment resulted in a change in the Arrhenius plot of phosphate transport to a biphasic one with a transition temperature of 30°C. This effect was not blocked by cycloheximide. The Arrhenius plots of glucose transport were triphasic and unchanged with vitamin D repletion. These data support a liponomic mechanism of action for $1,25(OH)_2D_3$ on phosphate transport.

Introduction

The mechanism of action of vitamin D on ion transport is unknown. Current evidence is supportive of two possibilities. The classic steroid hormone pathway in which vitamin D interacts with the cell nucleus to induce mRNA and protein synthesis is the first. The second, is a liponomic mechanism, whereby vitamin D directly modifies membrane lipid composition inducing altered ion transport. These possibilities are not necessarily mutually exclusive since vitamin D through its genomic actions, may induce the synthesis of enzymes which alter plasma membrane lipid metabolism. Studies of phosphate transport performed in the intestine have shown vitamin D to be stimulatory [1–3]. Some reports have suggested a mechanism of action related to altered membrane lipid composition [4–6] while others support a genomic effect [7]. Experiments studying phosphate transport by the kidney also support both genomic [8–10] and liponomic [11–14] effects for vitamin D.

Consideration of a liponomic effect of vitamin D is based on data demonstrating vitamin D induced changes in membrane lipid composition.

* To whom correspondence should be addressed.

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; 25(OH)D, 25-hydroxy vitamin D; $1,25(OH)_2D$, 1,25-dihydroxy vitamin D; Mes, 4-morpholineethanesulfonic acid, Tris, tris(hydroxymethyl)aminomethane; PC, phosphatidylcholine; PE, phospatidylethanolamine.

Motsumoto et al. [5] showed that intravenous $1,25(OH)_2D_3$ administered to vitamin D-deficient chicks 2 hours or less prior to study resulted in an increase in the phosphatidylcholine content and its degree of fatty acid unsaturation of the duodenal mucosa. These lipid effects were detectable in less than 1 hour and were not blocked by cycloheximide [5]. Evidence for lipid remodeling at the membrane level is provided by a $1,25(OH)_2D_3$-induced stimulation of phospholipase A_2 and lysophosphatidycholine acyltransferase activities in duodenal cells from rachitic rats [6].

Recent studies from this laboratory have demonstrated a change in renal brush-border membrane lipid composition during vitamin D deficiency which is partially corrected with physiologic repletion of $1,25(OH)_2D_3$ [11].

Vitamin D deficiency decreased membrane phosphatidylcholine (PC) and phosphatidylethanolamine content, and in the PC fraction of the phospholipid decreased stearic (18 : 0), linoleic (18 : 2) and arachidonic acid (20 : 4) levels. The palmitic acid (16 : 0) content of both phosphatidylcholine and phosphatidylethanolamine fractions was increased with vitamin D deficiency indicating that chain lengthening and desaturation of fatty acids in PC was impaired by the deficiency of vitamin D. Repletion with 30 pmol of $1,25(OH)_2D_3$ 16 hours prior to study increased membrane phosphatidylcholine content and also increased the levels of stearic, and linoleic acid in PC to normal.

Recent experiments where renal brush-border membrane vesicles were incubated with phosphatidylcholine liposomes containing cholesterol, 7-dehydrocholesterol, or cholecalciferol, showed that cholecalciferol was necessary in the liposomes to mediate the transfer of both itself and phosphatidylcholine to the brush-border membrane. This transfer resulted in an increase in brush-border membrane fluidity measured by electron spin resonance and a decrease in sodium-dependent glucose transport [13,14]. The authors speculated that vitamin D-3 in the liposomes induced fusion with the brush-border membrane vesicles suggesting another method for a vitamin D-induced effect through alterations of membrane lipid.

The following studies were performed to determine whether the vitamin D-induced changes in membrane lipid composition may mediate the physiologic effects of $1,25(OH)_2D_3$ on renal phosphate transport [8,15].

Materials and Methods

Experimental groups

A rat model of 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 [15]. These animals have normal levels of serum calcium and phosphorus, normal serum parathyroid hormone levels, undetectable serum 25(OH)D and reduced $1,25(OH)_2D$ levels. Normal rats were fed standard Purina rat chow containing 1% calcium and 0.6% phosphorus. Analysis of dietary calcium and phosphorus content was performed on samples ashed at 700°C in an oven for 72 h and reconstituted in 10% hydrochloric acid for assay.

The following groups of animals were studied. The first group was the vitamin D-deficient animals, the second group was vitamin D repleted by administering 15 pmol/100 g body weight of $1,25(OH)_2D_3$ intraperitoneally 24 h prior to study, a third group of vitamin D-deficient animals received 1.2 nmol/100 g body weight of $1,25(OH)_2D_3$ 3 h prior to study, the fourth and fifth groups of rats received cycloheximide 2 mg/kg, i.p., 4 h prior to study with or without $1,25(OH)_2D_3$, 1.2 nmol/100 g body weight 3 h prior to study and the sixth group were weight-matched normal animals.

Preparation and analysis of brush-border membrane vesicles

Rats were anesthetized with ether and blood drawn from the inferior vena cava prior to bilateral nephrectomy. The kidneys were immediately placed on ice and brush-border membrane vesicles prepared by a technique adapted from Beck and Sacktor [16] and previously reported for preparation of rodent kidneys from this laboratory [15]. Briefly, the kidneys were cleaned of any connective tissue, decapsulated, and sliced in half after which the medulla was dissected out

and discarded. The cortices were diced, weighed, and suspended in 50 mM mannitol, 2 mM Mes-Tris (pH 6.5) at a concentration of 20 ml/g tissue. The suspension underwent homogenization using a teflon pestle at 1000 rpm and then a polytron for three 20-s pulses at a power setting of 6. 1 M calcium chloride was added to give a final concentration of 10 mM and the suspension stirred for 20 min in a cold room. It was then spun for 15 min at $2000 \times g$ after which the supernatant was decanted and the pellet discarded. The supernatant was spun for 20 min at $35\,000 \times g$, the resulting supernatant was suctioned off, the pellet resuspended in 300 mM mannitol, 5 mM Mes-Tris (pH 6.5) and rehomogenized by hand with 6 strokes in a Dounce homogenizer. The process of discarding the supernatant and resuspending the pellet was repeated three more times to wash the vesicles. The final pellet was resuspended to achieve a final protein concentration of 20–30 $\mu g/\mu l$ and aliquoted into individual test tubes for transport experiments. Samples of the crude homogenate and vesicle preparation were saved for determination of protein content [17] and enzyme activities. We have previously characterized the enrichment of alkaline phosphatase activity, a marker of the brush border membrane and the decrease in enrichment of sodium potassium ATPase activity, a marker of the basolateral membrane in the brush-border membrane vesicles of the different experimental groups without evidence of intergroup variations [15].

Analysis of phosphate and glucose transport

Following preincubation of the brush-border membrane vesicles for one minute at specified temperatures, a solution used to initiate phosphate uptake was added containing 150 mM sodium chloride, 5 mM Mes-Tris (pH 6.5), and 0.625 mM phosphate (^{32}P, $25 \cdot 10^6$ cpm/ml). Transport studies were performed according to Aronson and Sacktor [18]. Transport was studied at 15 s of incubation, and stopped using a solution of ice-cold 150 mM sodium chloride, 5 mM Mes-Tris (pH 6.5), and 5 mM arsenate. Values for nonspecific retention of radioactivity on the filters were subtracted from the values obtained with vesicle samples. All points represent triplicate determinations. Uptakes were expressed in pmol/mg protein. Glucose transport was performed using a solution of 150 mM sodium chloride, 5 mM Mes-Tris (pH 6.5), and 1.6 mM glucose (D-[2(n)-^{3}H]glucose). Glucose transport was measured at 15 s and stopped with a solution of ice cold 150 mM sodium chloride and 5 mM Mes-Tris (pH 6.5).

Kinetic studies

The relationship between Na^+ gradient dependent P_i uptake and phosphate concentration was determined during incubations of 15 s at 30°C. Although an incubation time of 15 s does not represent perfect linearity it does correspond closely, for all temperatures studied, to the steep part of the ascending segment of a time-course curve. Furthermore, a number of laboratories have used either 15 s [19,20] or 20 s [21–23] incubations to determine the kinetic parameters of phosphate transport. The phosphate concentrations used ranged from 0.031 mM to 1 mM. Results were analyzed using Lineweaver-Burk plots.

Arrhenius plots of phosphate transport

The Arrhenius equation relates the rate constant for a reaction (k) to the activation energy of the reaction (E_a) and its temperature dependence (T),

$$k = A\, e^{-E_a/RT}$$

where A is a velocity constant (frequency factor) which encompasses an entropy term. This equation can be transformed to

$$\log k = \frac{-E_a}{2.3R}(1/T) + \log A$$

If the reciprocal of temperature is plotted against the $\log k$ then the slope is a measure of the activation energy of the reaction. This equation has been applied to the study of the temperature dependence of several processes including enzyme activities, transport processes, and receptor binding studies [24–27]. The plots generated are generally resolvable into either two or three lines with characteristic slopes representing the activation energies and transition temperatures calculated from the intersections of the lines. These parameters have been shown to be dependent on membrane fluidity which is largely a function of the mem-

brane lipid composition [26,28]. Previous work has been done applying this technique to phosphate uptake in renal brush-border membrane vesicles from normal animals [24] and has shown that both the K_m and V_{max} of phosphate uptake increase with temperature and that the Arrhenius plots demonstrated a break point at 23°C. Our studies utilized an analysis of the Arrhenius plots of phosphate uptake into brush-border membrane vesicles from normal, vitamin D-deficient, and vitamin D-replete animals to help elucidate potential liponomic mechanisms of action for $1,25(OH)_2D_3$.

The Arrhenius plots were constructed by measuring phosphate uptake at 15 of incubation using 0.625 mM phosphate. This concentration was chosen to produce maximal phosphate uptake at each temperature and thereby minimize any effect of temperature on the K_m of the plots. Sixteen to twenty temperatures were studied between 8°C and 42°C. Potassium gradient stimulated phosphate uptake was also studied and subtracted from total phosphate uptake to give sodium gradient dependent phosphate transport. The Arrhenius plots of glucose uptake were performed as controls. The data was analyzed using computer generated plots. The best two-line and three-line fits were determined by using the method of least squares and by calculating the minimum residual sum of squares for every two or three line possibility. The *F*-test was used to compare the two-line fit to a three-line fit [29].

Materials

^{32}P, orthophosphoric acid (carrier free) and D-[2-^{3}H]glucose were obtained from New England Nuclear (Boston, MA). Other chemicals were of the highest purity available from commercial sources. All solutions were filtered through a 0.45 μm Millipore filter the day of the experiment.

Results

Kinetics

The results of the kinetic analysis of Na^+ gradient dependent P_i uptake in brush-border membrane vesicles are shown in Fig. 1. After 24 h of repletion of vitamin D deficient rats using 12.5 ng (15 pmol/100 g body weight) of $1,25(OH)_2D_3$, there was a significant stimulation of the V_{max} of

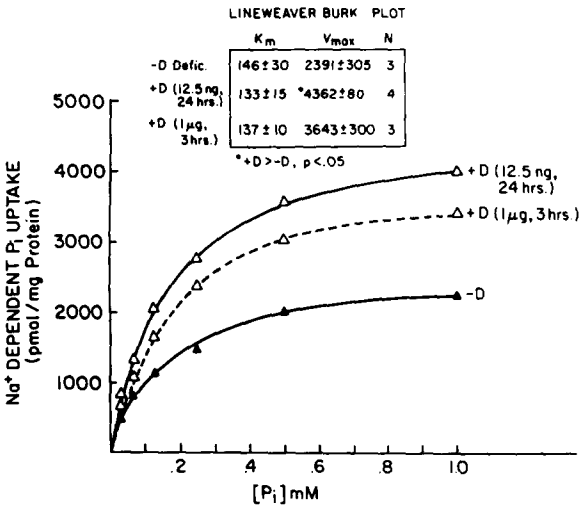

Fig. 1. Kinetic analysis of sodium-dependent P_i uptake into brush-border membrane vesicles from vitamin D-deficient (−D) and vitamin D-replete (+D) rats. Uptakes are expressed as pmol phosphate/mg protein per 15 s at 30°C, and performed as described in Methods. The inset shows an analysis of the data using Lineweaver-Burk plots. Significance, *+D > −D, $p < 0.05$, $N = 4$ for each group.

phosphate transport from 2391 ± 305 to 4362 ± 80 pmol/mg protein per 15 s with no change in the K_m. A dose of 1.2 nmol/100 g body weight, 3 h prior to study did increase the V_{max}, but this did not reach statistical significance.

Arrhenius plots of phosphate transport

Representative examples of Arrhenius plots of sodium dependent phosphate uptake into brush-border membrane vesicle from normal, vitamin D-deficient and 24 h-vitamin D-replete (15 pmol/100 g body weight of $1,25(OH)_2D_3$) animals are shown in Figs. 2 and 3. Fig. 2 shows a representative Arrhenius plot using brush-border membrane vesicles prepared from normal animals. These plots were best resolved into three lines with slopes of -0.2 ± 0.2, -4.2 ± 0.4, and -9 ± 0.5 (Table I) which represent activation energies of 0.4, 8.4, and 17.8 kcal/mol per Kelvin, respectively. The intersections of the lines represent the two transition temperatures of 23.4 ± 0.4 and 35.5 ± 1.9°C (Table I). The *F*-test comparing the best three-line fit to the best two-line fit was significant ($p < 0.05$) favoring the three-line fit in all instances. It can be seen from Fig. 3 that sodium-de-

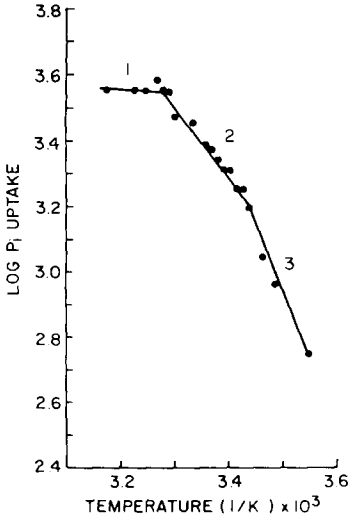

Fig. 2. An Arrhenius plot of Na^+-dependent phosphate transport into brush-border membrane vesicles prepared from normal animals. Transport was performed using 0.625 mM phosphate at 15 s of incubation as described in Methods. The plots are best resolved into three lines (1, 2, 3) with two transition temperatures.

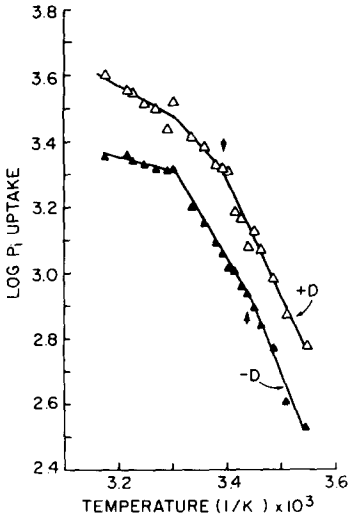

Fig. 3. Arrhenius plots of Na^+-dependent phosphate transport into brush-border membrane vesicles prepared from vitamin D-deficient (−D) and -replete (+D) animals. Replete animals received 15 pmol/100 g body weight of $1,25(OH)_2D_3$ 24 h prior to study. Transport was performed using 0.625 mM phosphate at 15 s of incubation.

pendent phosphate transport was greater into brush-border membrane vesicles from vitamin D-replete animals at all temperatures studied. Table I shows a tabulation of the slopes and transition temperatures of the plots for the three groups. Vitamin D repletion increased the lower transition temperature from 18 ± 0.7°C to 23.5 ± 0.9°C and decreased the slopes of the second and third lines from -5.8 ± 0.2 to -4.5 ± 0.4 and from -10.2 ± 0.4 to -7.7 ± 0.3, respectively. These changes in the slopes represent decreases in the activation energy for sodium-dependent phosphate transport from 11.5 and 20.4 kcal/mol per K to 8.9 and 15.4 kcal/mol per K, respectively. There were no significant differences between the higher transition

TABLE I

SUMMARY OF THE SLOPES AND TRANSITION TEMPERATURES CALCULATED FROM THE ARRHENIUS PLOTS OF SODIUM-DEPENDENT PHOSPHATE TRANSPORT

M_1, M_2, and M_3 represent the slopes of the lines (1,2,3); T_1 and T_2 are the transition temperatures calculated from the intersections of the lines. Results are expressed as $\bar{X} \pm$ S.E., significance: * $p < 0.02$, ** $p < 0.05$.

	Normal ($N = 3$)	D-deficient ($N = 5$)	D-replete ($N = 3$) (15 pmol/100 g body wt. per 24 h)
M_1	-0.2 ± 0.2	-0.8 ± 0.3	-1.1 ± 0.5
M_2	-4.2 ± 0.4 *	-5.8 ± 0.2	-4.5 ± 0.4 **
M_3	-9.0 ± 0.5	-10.2 ± 0.4	-7.7 ± 0.3 *
T_1 (°C)	35.5 ± 1.9	32.9 ± 1.3	$32.5 \pm .8$
T_2 (°C)	23.4 ± 0.4 *	18.0 ± 0.7	23.5 ± 0.9 *

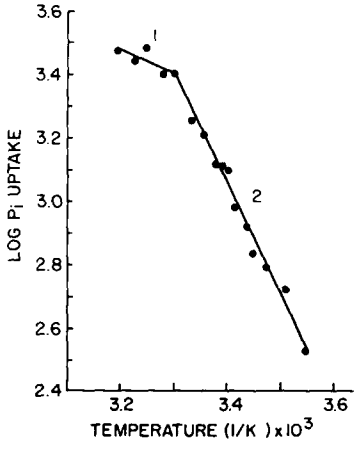

Fig. 4. An Arrhenius plot of Na^+-dependent phosphate transport into brush-border membrane vesicles prepared from vitamin D-replete rats who received 1.2 nmol/100 g body weight of $1,25(OH)_2D_3$ 3 h prior to study. Transport was performed using 0.625 mM phosphate at 15 s of incubation. These plots were best resolved into two lines (1, 2) with one transition temperature.

TABLE II

SUMMARY OF THE SLOPES AND TRANSITION TEMPERATURES OF THE ARRHENIUS PLOTS OF SODIUM-DEPENDENT PHOSPHATE TRANSPORT FOLLOWING 3 h OF HIGH DOSE $1,25(OH)_2D_3$ REPLETION

M_1 and M_2 represent the slopes of the lines (1, 2) and T is the transition temperature calculated from the intersection of the lines. Individual experiments are listed per each group.

Expt.	M_1	M_2	T
A. $1,25(OH)_2D_3$ (1.2 nmol/100 g body weight per 3 h)			
1	−1.6	−6.6	30.8
2	−1.7	−8.8	28.8
3	−1.7	−7.9	30.4
B. $1,25(OH)_2D_3$ (1.2 nmol/100 g body weight per 3 h) + cycloheximide (2 mg/kg per 4 h)			
1	−0.3	−6.7	29.3
2	−1.8	−6.6	31.1

temperatures or the slopes of the first lines. The observed changes in the slopes and transition temperatures with vitamin D repletion produced plots with comparable slopes and transition temperatures to those derived from the plots of normal animals.

An Arrhenius plot of Na^+-dependent P_i transport into brush-border membrane vesicles from vitamin D-replete rats following a dose of 1.2 nmol/100 g body weight of $1,25(OH)_2D_3$ 3 h prior to study is shown in Fig. 4. These plots were best resolved into two lines with a transition temperature of 30 ± 0.6°C and slopes of -1.7 ± 0.1 and -7.8 ± 0.6 representing activation energies of

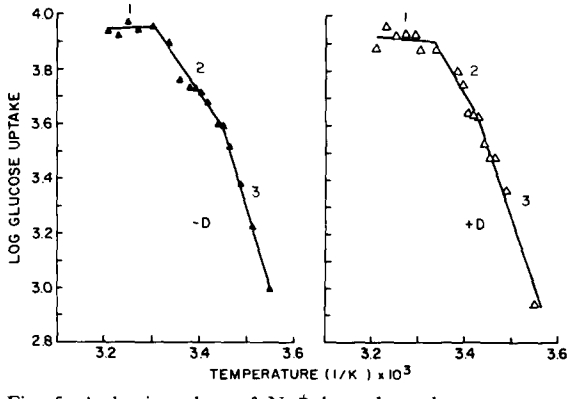

Fig. 5. Arrhenius plots of Na^+-dependent glucose transport into brush-border membrane vesicles from vitamin D-deficient (−D) and -replete (15 pmol/100 g body weight per 24 h, +D) rats. Transport was performed using 1.6 mM glucose and measured at 15 s of incubation.

TABLE III

SUMMARY OF THE SLOPES AND TRANSITION TEMPERATURES CALCULATED FROM THE ARRHENIUS PLOTS OF SODIUM-DEPENDENT GLUCOSE TRANSPORT

M_1, M_2, M_3, T_1 and T_2 are defined in Table I. There were no significant differences between the groups. Results are expressed as $\bar{X} \pm$ S.E.

	D-deficient ($N = 3$)	D-replete ($N = 3$) (30 pmol/24 h)
M_1	0	-0.1 ± 0.1
M_{2+}	-4.6 ± 0.9	-5.4 ± 0.7
M_3	-9.7 ± 1.8	-11.0 ± 0.4
T_1 (°C)	29.3 ± 0.7	27.0 ± 0.4
T_2 (°C)	18.0 ± 2	18.5 ± 0.5

3.4 and 15.5 kcal/mol per K, respectively (Table II). Pretreatment of the animals with cycloheximide did not block this effect; the plots were also best resolved into two lines with one transition temperature of 30°C. The F test favored a two-line fit as the best in both the 1.2 nmol and the cycloheximide plus 1.2 nmol treatment groups ($p < 0.05$). Cycloheximide treatment alone resulted in a three-line plot unchanged from that of vitamin D deficiency.

Arrhenius plots of glucose transport

Fig. 5 shows representative examples of Arrhenius plots of sodium dependent glucose uptake into brush-border membrane vesicles from 24 h vitamin D-replete and vitamin D-deficient rats. These plots were triphasic with transition temperatures of approximately 18°C and 28°C. Table III shows a tabulation of the slopes and transition temperatures of the Arrhenius plots of glucose transport. There were no significant differences in these plots when compared between the treatment groups.

Discussion

Previous studies from this laboratory and others [8,12,15,30–33] have confirmed the stimulatory role for $1,25(OH)_2D_3$ on renal phosphate transport. In addition similar $1,25(OH)_2D_3$-induced membrane lipid changes have been documented in both the intestine [5] and renal brush-border membrane [11]. The studies reported here were performed to

determine the functional significance of the membrane lipid changes in regard to phosphate transport. Such a correlation provides evidence for a relationship between alterations in membrane lipid produced by $1,25(OH)_2D_3$ and the stimulation of Na^+-dependent phosphate transport into renal brush-border membrane vesicles.

A number of cellular processes including enzyme activities, transport processes, and receptor functions have been shown to be lipid dependent [25]. It is theorized that changes in membrane lipid composition will alter membrane fluidity either in a generalized fashion or confined to a microdomain, and result in changes in the processes listed above. Several methods have been used to alter membrane fluidity experimentally and evaluate its functional effects. Cholesterol has been used to alter membrane fluidity and modulate calcium-ATPase activity in the sarcoplasmic reticulum [34]. Filipin, a polyene antibiotic, which increases membrane fluidity has been shown to increase calcium but not phosphate transport into intestinal brush border membrane vesicles prepared from vitamin D-deficient but not vitamin D-treated chicks [1,35]. The methyl esters of *cis*- and *trans*-vaccenic acid have been used to alter both membrane lipid composition and fluidity [36]. Treatment of duodenal brush-border membrane vesicles from vitamin D-deficient chicks with *cis*-vaccenic acid has been shown to increase calcium transport while treatment of brush-border membrane vesicles from vitamin D-replete animals with *trans*-vaccenic acid has been shown to decrease calcium transport; no effects were reported on either glucose or phosphate transport [37]. Another method used to alter membrane fluidity has been temperature. Construction and analysis of Arrhenius plots for enzyme activities and transport processes have been used to evaluate the functional significance of alterations in membrane lipid composition [25,26,29,38,39].

Arrhenius plots of P_i transport were constructed to evaluate the relationship between vitamin D-induced changes in membrane lipid composition and phosphate transport. Repletion with 15 pmol/100 g body weight of $1,25(OH)_2D_3$ 24 h prior to study resulted in an increase in the V_{max} of phosphate transport at all temperatures in addition to the increase in the lower transition temperature and decrease in the slopes (activation energies) of the second and third lines. The temperature-independent increase in phosphate transport could be due to either an increase in the absolute number of carrier units or an increase in the availability of carrier units secondary to membrane lipid changes. The transition temperatures and slopes are lipid-dependent parameters [25] which are preserved even after the lipids are extracted and liposomes prepared [26,28]. The changes in the slopes and transition temperatures reflect a lipid dependent effect of $1,25(OH)_2D_3$ to facilitate sodium-dependent phosphate transport. The effects of $1,25(OH)_2D_3$ are specific for the Arrhenius plots of phosphate as opposed to glucose transport, even though glucose transport has previously been shown to have a lipid dependence in other membranes [27,40,41] and to vary with changes in membrane phosphatidylcholine and phosphatidylethanolamine content [40]. Since it is possible that the membrane lipid changes induced by vitamin D are located in the lipid domain of the phosphate carrier protein, the different transition temperatures for the Arrhenius plots of phosphate transport as opposed to glucose transport provide further support that these plots reflect specific local changes in membrane lipid composition, rather than a global phase transition of the membrane. The lower transition temperature of 23°C in the Arrhenius plot of phosphate transport differs from that obtained when phosphate transport was studied in brush-border membrane vesicles derived from hogs [38]. One possible explanation would be a species difference. The transition temperature for the Arrhenius plot of alkaline phosphatase activity was 12.4°C in the hog membranes [38], as opposed to 26°C in membranes derived from dog kidneys [26] and 34.7°C in those from rats [42]. Another explanation might be that our studies were performed in the presence of a sodium gradient, as opposed to sodium equilibrium. Previous studies performed under sodium-equilibrated conditions demonstrated a similar and non-specific temperature dependence for glucose and phosphate transport and alkaline phosphatase activity [38]. It is possible that the Arrhenius plots of phosphate transport are different in the presence or absence of a sodium gradient. Under the more physiologic conditions, we were able to demon-

strate specific differences between the plots of glucose transport and phosphate transport in addition to an effect of vitamin D which could reflect a lipid micro-domain. Our transition temperature of 23°C is the same as that previously reported in brush-border membrane vesicles from normal rats [24]. The effect of vitamin D to increase the lower transition temperature is difficult to explain in view of the fact that vitamin D has been shown to increase the phosphatidylcholine content and the degree of unsaturation of the fatty acid components of phosphatidylcholine; both changes which would tend to decrease the transition temperature. However, the dependency of transition temperature is much more complex than currently understood as are the effects of vitamin D on membrane lipids.

Incorporation of vitamin D into the membrane [13,14] may also play a role in determining the transition temperature. The treatment of the rats with a pharmacologic dose of 1.2 nmol of $1,25(OH)_2D_3$/100 g body weight three hours prior to study had a different effect on the Arrhenius plot of phosphate transport than the physiologic repletion studies. Arrhenius plots after higher dose, 3 h treatment, were best resolved into two lines whose slopes are similar to the first and third line of the triphasic plots generated from normal animals or low dose vitamin D replete animals (15 pmol/100 g body weight of $1,25(OH)_2D_3$). The loss of the middle line, which represented a facilitation of the sodium dependent phosphate transport with a decrease in the energy of activation for this process was consistent with the smaller stimulation of the V_{max} of phosphate transport using this protocol at 30°C. In addition, this effect was not blocked by cycloheximide pre-treatment, suggesting a direct membrane effect, possibly an insertion of vitamin D itself into the membrane.

In summary, the studies reported here support a modulation of renal P_i transport by $1,25(OH)_2D_3$ related to changes in membrane lipid composition. However, the mechanisms by which $1,25(OH)_2D_3$ induces these membrane lipid alterations are probably multiple. Pharmacologic treatment with 1.2 nmol of $1,25(OH)_2D_3$/100 g body weight three hours prior to study resulted in a direct membrane effect which was not blocked by cycloheximide. However, physiologic repletion of vitamin D with 15 pmol/100 g body weight of $1,25(OH)_2D_3$ 24 hours prior to study revealed different changes in the Arrhenius plots suggesting effect differing from the cycloheximide-resistant effects of acute pharmacologic treatment.

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