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MECHANISMS OF CALCIUM TRANSPORT IN SMALL INTESTINE

Progress Report

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Abstract

Low phosphorus diets have been shown to stimulate intestinal calcium transport by stimulating the production of 1,25-dihydroxyvitamin D₃ in the kidney, revealing that serum inorganic phosphorus and hence renal cortex levels of inorganic phosphorus are an important regulator of the synthesis of 1,25-dihydroxyvitamin D₃, the active form of the vitamin in stimulating intestinal calcium transport. It had been previously shown that hypocalcemia, bringing about secretion of parathyroid hormone, also results in stimulation of 1,25-dihydroxyvitamin D₃ production. The latter is completely mediated by the parathyroid hormone. Thus both hypophosphatemia and parathyroid hormone trigger the synthesis of 1,25-dihydroxyvitamin D₃ by an unknown mechanism.

Ethane-1-hydroxy-1,1-diphosphonate (EHDP) but not dichloro-diphosphonate causes rickets and reduced intestinal calcium absorption. The reduced intestinal calcium absorption is completely corrected by administration of 1,25-dihydroxyvitamin D₃ and furthermore EHDP can be shown to block production of 1,25-dihydroxyvitamin D₃ by inhibiting the enzyme itself, thus solving the question of how the EHDP inhibits intestinal calcium absorption.

The biological activity of the variety of metabolites and analogs of vitamin D has been examined. The most potent is 1,25-dihydroxyvitamin D₃ given parenterally. Under these circumstances the compound is 10 times more active than vitamin D₃ in all the systems tested. 1 α -Hydroxyvitamin D₃ and 25-hydroxyvitamin D₃ are both five times more effective than vitamin D₃ in the rat. 24,25-Dihydroxyvitamin D₃ stimulates only intestinal calcium transport in the rat and to do so it must be converted to 1,24,25-trihydroxyvitamin D₃. The latter compound is almost as effective as 1,25-dihydroxyvitamin D₃ in stimulating intestinal calcium transport, but possesses little or no activity on the mobilization of calcium from bone and the stimulation of phosphate transport reactions.

Work on the mechanism of intestinal calcium transport has centered around the isolation of receptor proteins for 1,25-dihydroxyvitamin D₃ and the isolation of two proteins from brush border which appear to be involved in intestinal calcium transport. Two receptor proteins have been worked with, one a cytoplasmic protein and the other a protein from the nuclei debris fraction. The latter appears to be highly specific for 1,25-dihydroxyvitamin D₃ when it is given in vivo. The two proteins of brush border involved in calcium transport are being purified to homogeneity.

I. Mechanism of Intestinal Calcium Transport

(A) During the past year considerable effort has been expended in an attempt to obtain in homogeneous form two proteins of brush border which are altered by the administration of 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃). In the brush borders of vitamin D-deficient chick intestine a protein of approximate molecular weight of 200,000 is present and is absent from similar preparations from vitamin D-supplemented chicks. Administration of 1,25-(OH)₂D₃ to chicks results in a disappearance of this protein and the appearance of another protein of molecular weight 230,000. This change exactly correlates with intestinal calcium transport response to this active form of vitamin D. Highly purified preparations of both proteins have been obtained in small quantities. The 200,000 M.W. protein does not bind calcium while the 230,000 M.W. protein does. Both possess alkaline phosphatase activity while preliminary experiments show that the 230,000 M.W. protein has a calcium ATPase activity. Attempts to scale up purification have failed for unknown reasons and Dr. Moriuchi, who was carrying out the project, completed her stay in the U. S. and has returned to Japan. Dr. Robert Reynolds has taken over the project and is attempting to obtain large amounts of these proteins for more complete biochemical and physical studies. It seems likely that these proteins are intimately involved in the intestinal calcium transport mechanism and will be given a high priority in investigation.

(B) Work has continued on the receptor proteins for 1,25-(OH)₂D₃ in intestine. A cytoplasmic 1,25-(OH)₂D₃ binding protein has been studied but it lacks either tissue specificity or specificity for 1,25-(OH)₂D₃. A nuclear fraction receptor has also been studied and shown to have considerable specificity for 1,25-(OH)₂D₃ given in vivo but not added in vitro. Attempts to develop in vitro specificity are now in progress. In any case the nuclear-debris receptor (low density) protein has been highly purified but is unstable. Dr. Charles Frolik has now left the laboratory and Dr. Joyce Knutson has taken over this project. The switchover to new personnel has slowed progress somewhat. This area will likely be an active one in the future and will be emphasized in our future studies.

II. Regulation of Intestinal Calcium Transport by Regulation of 1,25-(OH)₂D₃ Biosynthesis

The mechanism whereby ethane-1-hydroxy-1,1-diphosphonate (EHDP) inhibits intestinal calcium absorption has now been clearly shown to be due to an inhibition of 1,25-(OH)₂D₃ synthesis. This has further been shown to be due to a direct inhibition of 25-OH-D₃-1-hydroxylase by the diphosphonate. Dichlorodiphosphonate has much less effect both on the 1-hydroxylase and in vivo calcium transport. These studies have also revealed that vitamin D probably plays a role in diphosphate uptake by kidney tissue.

The well-known stimulation of calcium transport by low phosphorus diets is due to the stimulation of $1,25-(\text{OH})_2\text{D}_3$ synthesis by hypophosphatemia. Renal cortex levels of inorganic phosphorus apparently regulate synthesis of $1,25-(\text{OH})_2\text{D}_3$. This is controlled by serum phosphorus and by the parathyroid hormone block of renal reabsorption of inorganic phosphorus. Thus physiologically parathyroid hormone on one hand and low blood phosphorus on the other stimulate $1,25-(\text{OH})_2\text{D}_3$ production. The molecular mechanism of this regulation remains unsolved and is being studied under support by my NIH grant.

III. Development of Intestinal Calcium Transport Process in Chick Embryo

A comparative study of the development of intestinal calcium transport in the chick embryo and the development of enzymes which hydroxylate vitamin D has been completed. It now seems clear that calcium binding protein does not appear until the day after hatching in chicks. This is in agreement with work from Wasserman's laboratory. However, the existence of the vitamin D_3 -25-hydroxylase in embryos can be shown both in vitro and in vivo as early as 13 days in embryonic life. Furthermore, the 1-hydroxylase of kidney appears before hatching and a complete study of in vivo appearance of $1,25-(\text{OH})_2\text{D}_3$ and $25-\text{OH}-\text{D}_3$ in chick embryo has been carried out. In short these metabolites appear in sufficient time to bring about production of calcium binding protein and intestinal calcium transport. However, the intestine apparently is not responsive to the $1,25-(\text{OH})_2\text{D}_3$ until the day after hatching. Several possibilities exist. It is possible that the receptor protein for $1,25-(\text{OH})_2\text{D}_3$ does not appear in intestine until that time. Another possibility is that the receptor proteins are present but the mechanism for stimulating calcium binding protein and other proteins involved in intestinal calcium transport do not make their appearance. This will be investigated in the future, but will not receive a high priority at this time.

IV. The Biological Testing of Analogs and Metabolites of Vitamin D

The biological testing of analogs and metabolites of vitamin D has continued. The complete biological profile on 1α -hydroxyvitamin D_3 ($1\alpha\text{-OH}-\text{D}_3$), the synthetic analog of $1,25-(\text{OH})_2\text{D}_3$, has now been completed. It is equally effective whether given orally or parenterally; this contrasts with $1,25-(\text{OH})_2\text{D}_3$, which is much more effective when given parenterally at least on a chronic basis. When given parenterally the $1\alpha\text{-OH}-\text{D}_3$ is about five times more effective than vitamin D_3 in the prevention of rickets, stimulation of intestinal calcium transport, mobilization of calcium from bone and in the elevation of serum inorganic phosphorus. On the other hand, $1,25-(\text{OH})_2\text{D}_3$ given parenterally is approximately 10 times more effective than vitamin D_3 in these parameters. $25-\text{OH}-\text{D}_3$ appears to be equal in activity to the $1\alpha\text{-OH}-\text{D}_3$. The difference between these two compounds is that $1\alpha\text{-OH}-\text{D}_3$ is fully effective in nephrectomized animals whereas $25-\text{OH}-\text{D}_3$ is not.

The complete profile of the biological activity of 24,25-dihydroxyvitamin D_3 ($24,25-(\text{OH})_2\text{D}_3$) has also been carried out and this compound is shown to act in vitamin D-deficient animals by stimulating intestinal calcium transport. To do this

it must be converted to another metabolite which has been isolated in pure form in our laboratory and identified as 1,24,25-trihydroxyvitamin D₃ (1,24,25-(OH)₃D₃). This compound is almost as effective as 1,25-(OH)₂D₃ in stimulating intestinal calcium transport but it possesses no activity in bone calcium mobilization nor does it bring about an elevation of serum inorganic phosphorus. Continued examination of biopotent compounds on intestinal calcium transport will continue.

V. Effect of *Solanum malacoxylon* on Intestinal Calcium Transport

The plant *Solanum malacoxylon* causes a severe calcifying disease in cattle in South America. Extracts of this plant have been shown to elevate serum calcium concentration and stimulate intestinal calcium absorption. We have studied extracts of this plant and have shown that it is very much like 1,25-(OH)₂D₃ in stimulating intestinal calcium transport, but unlike vitamin D₃ it does not stimulate mobilization of calcium from bone. Furthermore, its effect is not additive to that of vitamin D₃ on intestinal calcium transport which suggests that they affect the same system in the intestine. A log dose response curve has been developed so that we may assay quantitatively the active component of this extract. We are currently attempting isolation and identification of the compound.

VI. Transport of Inorganic Phosphate in Intestine

Interest in this has been developed since it has now been shown that intestinal phosphate transport is responsive to 1,25-(OH)₂D₃. It is now clear that this transport process is quite independent of the transport process for calcium in the duodenum. We have demonstrated that this process is specifically stimulated by 1,25-(OH)₂D₃ and in agreement with previous work from other laboratories, the process requires the presence of an oxidizable substrate such as glucose but does not require the presence of calcium in the incubation medium. Nephrectomy prevents the response of this system to 25-OH-D₃ but does not prevent the response to the 1,25-(OH)₂D₃. Work is continuing on the phosphate transport mechanism of intestine and in other tissues which are now known to be responsive to 1,25-(OH)₂D₃.

Papers Published Under This Contract

- COO-1668-48--DeLuca, H.F. Vitamin D Metabolites in Medicine and Nutrition in "Proceedings of the 1973 Georgia Nutrition Conference for the Feed Industry" (February 14-16, 1973). University of Georgia, Atlanta, Georgia, pp. 88-108 (1973).
- COO-1668-49--Bonjour, J.-P., H.F. DeLuca, H. Fleisch, U. Trechsel, L.S. Matejowec, and J.L. Omdahl. Reversal of the EHDP Inhibition of Calcium Absorption by 1,25-Dihydroxycholecalciferol. *Europ. J. Clin. Invest.* 3, 44-48 (1973).
- COO-1668-50--Tanaka, Y., and H.F. DeLuca. The Control of 25-Hydroxyvitamin D Metabolism by Inorganic Phosphorus. *Arch. Biochem. Biophys.* 154, 566-574 (1973).
- COO-1668-51--Omdahl, J.L., and H.F. DeLuca. Regulation of Vitamin D Metabolism and Function. *Physiol. Rev.* 53, 327-372 (1973).
- COO-1668-52--Boyle, I.T., J.L. Omdahl, R.W. Gray, and H.F. DeLuca. The Biological Activity and Metabolism of 24,25-Dihydroxyvitamin D₃. *J. Biol. Chem.* 248, 4174-4180 (1973).
- COO-1668-53--Tanaka, Y., H. Frank, and H.F. DeLuca. Intestinal Calcium Transport: Stimulation by Low phosphorus Diets. *Science* 181, 564-566 (1973).
- COO-1668-54--Chen, T.C., and H.F. DeLuca. Receptors of 1,25-Dihydroxycholecalciferol in Rat Intestine. *J. Biol. Chem.* 248, 4890-4895 (1973).
- COO-1668-55--DeLuca, H.F., and A. Kleiner-Bossaller. Metaboliten von Vitamin D als Hormone in der Regulation des Calcium- und Phosphatstoffwechsels. *Mschr. Kinderheilk.* 121, 329-337 (1973).
- COO-1668-56--DeLuca, H.F. Regulation of Functional Vitamin D Metabolism: A New Dimension in Calcium Homeostasis, in "Cornell Seminars in Nephrology", (E. Lovell Becker, ed.) pp. 47-71 (1973).
- COO-1668-57--DeLuca, H.F. Vitamin D-Overview: Regulation of Functional Vitamin D Metabolism: A New Endocrine System Involved in Calcium Homeostasis, in "Calcified Tissues" (H. Czitober and J. Eschberger, eds.) pp. XXIX-XXXVIII (1972).

COO-1668-58--Bhattacharyya, M.H., and H.F. DeLuca. Subcellular Location of Rat Liver Calciferol-25-Hydroxylase. Arch. Biochem. and Biophys. 160, 58-62 (1974).

COO-1668-59--Seymour, J.L., and H.F. DeLuca. Action of 25-Hydroxy-dihydrotachysterol₃ on Calcium Metabolism in Normal and Thyroparathyroidectomized Rats. Endocrinology 94, 75-81 (1974).

COO-1668-60--Holick, M.F., and H.F. DeLuca. Vitamin D Metabolism. Ann. Rev. Med. (in press).

COO-1668-61--DeLuca, H.F. Vitamin D--1973. Am. J. of Med. (in Press). (Proceedings of the Third F. Raymond Keating Memorial Symposium, September 10-12, 1973).

Proposed Technical Scope

Two large molecular weight proteins which are believed to be involved in the response of the intestine to vitamin D will be isolated in pure form in sufficient quantities to permit detailed biochemical and physical estimations of their activity and binding capacities for different divalent cations. One of these proteins is found in the microvilli of vitamin D-deficient animals while the other protein appears in the microvilli of chicks given vitamin D. Disappearance of the protein in the case of the -D animals following 1,25-dihydroxyvitamin D₃ treatment is correlated with the appearance of the larger molecular weight protein. It seems likely that these proteins are involved in the intestinal calcium transport response to the 1,25-dihydroxyvitamin D₃.

A continuation of the study of factors which affect calcium transport of intestine by affecting metabolism of vitamin D in the kidney will be continued. Of special importance will be a study of the role of the diphosphonates and pyrophosphate on 25-hydroxyvitamin D₃-1 α -hydroxylase of the kidney. In addition, the mechanism whereby low phosphorus diets and parathyroid hormone stimulate production of 1,25-dihydroxyvitamin D₃ will be emphasized.

The study of metabolites and analogs of vitamin D which are isolated or prepared under other support in this laboratory will be examined for their effects on intestinal calcium transport.

Finally, major efforts will be placed on the pursuit of an understanding of the molecular mechanism of intestinal calcium transport in response to 1,25-dihydroxyvitamin D₃.

Proposed Technical Program

During the past year Dr. Sachiko Moriuchi, who has been responsible for work on the intestinal calcium transport mechanism especially the proteins of microvilli, has now returned to Japan and she has been replaced by Dr. Robert Reynolds, who is continuing primarily the work on the brush border proteins. Besides him, Mrs. Lee Ann Baxter, a graduate student, is working on the effect of diphosphonates and pyrophosphate on vitamin D metabolism and intestinal calcium transport. She is supported by funds provided by Procter & Gamble because of their interest in the diphosphonates. Dr. Yoko Tanaka is supported on my NIH program-project grant dealing with the fat-soluble vitamins, but much of her work is concerned with the regulation of intestinal calcium transport. Finally, a student on the training grant provided to our department by the NIH, Mr. Martin Ribovich, has been studying adaptation of intestinal calcium transport to dietary calcium and the role of vitamin D metabolism in this regulation. During the next year it is anticipated that all of these individuals will continue to function in the proposed program. I must emphasize, however, that the funds provided by the AEC are only sufficient to provide supplies and a salary for one postdoctorate. Progress could be rapidly accelerated in the intestinal calcium transport area if some funds were provided to make up for the loss in purchasing capacity of the small grant we have held at the same level for the past six years.

The major efforts will be directed toward elucidating the molecular mechanism of intestinal calcium transport and especially the mechanism whereby 1,25-dihydroxy-vitamin D₃ (1,25-(OH)₂D₃) initiates this process. It has been accepted in many laboratories that the primary effect of the 1,25-(OH)₂D₃ is to alter the brush border surface of the intestine so that it may now transport calcium. Beyond that agreement there is much uncertainty as to what mechanism takes place. Wasserman and his colleagues have isolated calcium binding protein, M.W. 24,000, which appears to play some role in intestinal calcium absorption. However, its role is yet to be defined. Since 1,25-(OH)₂D₃ will initiate intestinal calcium transport within 3-6 hours after its administration, we have been examining by disc-gel electrophoresis the protein components of the brush border during this response to the 1,25-(OH)₂D₃ compound. Using either urea gels or sodium dodecyl sulfate gels we can show that in the D-deficient brush borders there is a large molecular weight protein (200,000 approximately) which is absent from the profiles found from animals given vitamin D. Upon administration of 1,25-(OH)₂D₃ to the D-deficient chicks, this protein disappears from the profiles and there appears instead a protein of slower migration and a molecular weight of approximately 230,000. We have been able to solubilize these proteins and have been able to highly purify them. Preliminary studies revealed that the protein isolated from brush borders of chicks given 1,25-(OH)₂D₃ possesses calcium binding activity, calcium ATPase activity and alkaline phosphatase activity, whereas that isolated from the -D mucosa possesses only alkaline phosphatase activity. The appearance and disappearance of this protein correlates exactly with the intestinal calcium transport response to the 1,25-(OH)₂D₃. We are, therefore, excited about these proteins as playing a major role in intestinal calcium transport, at least in response to

vitamin D. By the use of gel filtration chromatography and diethylaminoethyl Sephadex chromatography we have been able to highly purify these proteins. However, the amount we have obtained is extremely small. We have tried to scale-up operations and so far met with little success. We are continuing our attempts to isolate these proteins in pure form so that we may study in detail their calcium binding activity and their ability to bind other radionuclides including radium, polonium, beryllium, lead, mercury, cadmium, etc. Furthermore, we would like to learn what its composition is in terms of lipid and amino acids. We would like to learn more about its physical properties using sedimentation methods, diffusion methods, etc. We would also like to study various enzymatic activities besides alkaline phosphatase and ATPase activity. Finally, we would like to incorporate these proteins into black lipid membranes to learn if they change the properties of the membrane in relation to conductivity in the presence of calcium versus other ions such as sodium, potassium, etc.

The second largest emphasis will be placed on the isolation of receptor proteins for $1,25-(OH)_2D_3$ in mucosa. We know that there are two proteins that one can work with. The first is the cytoplasmic protein which does bind $1,25-(OH)_2D_3$ but has very little specificity for this compound. It may, however, be that this initial receptor protein may be altered only when $1,25-(OH)_2D_3$ is attached to it. We propose to isolate the cytoplasmic protein to a high degree of purity and study its incubation with intestinal nuclei or other fractions to determine if we can show a high degree of specificity in the transfer of this protein + $1,25-(OH)_2D_3$ to chromatin, etc. Finally, we would like to isolate the receptor protein in the nuclear-debris fraction following in vivo injection of radioactive $1,25-(OH)_2D_3$. Although we have purified this protein we have found it to be very unstable. Attempts to stabilize it with glycerol, ionic strength, and other means have not yet been successful. We would like to continue this important project since we feel these proteins may play a central role in the expression of $1,25-(OH)_2D_3$ function in intestinal calcium transport.

Besides attempted isolation of the receptor proteins we would like to carry out experiments similar to those carried out in the estrogen field in which the cytoplasmic protein is converted to a somewhat different protein during passage into the nuclear portion of the cell. Finally, we have synthesized very high specific activity 25-hydroxyvitamin D_3 ($25-OH-D_3$) which will permit for the first time the generation of radioactive $1,25-(OH)_2D_3$ of high specific activity. This material will be used for autoradiography and if possible microautoradiography in conjunction with Dr. Matthews at Baylor and Dr. Giulia Witmer in Paris to try to determine the subcellular location of the $1,25-(OH)_2D_3$ while it is carrying out its function in stimulating intestinal calcium transport. Our role in this work will be merely to supply the high specific activity $1,25-(OH)_2D_3$ and the deficient animals. We will inject the radioactive material and determine when the intestine has responded to the material. We will kill the animals at various times after the administration of radioactive $1,25-(OH)_2D_3$ to determine intestinal calcium transport. The tissues will be fixed according to the direction of these individuals and sent to them for autoradiography.

The third priority item will be continuation of the study of analogs and metabolites of vitamin D and their effect on intestinal calcium transport. We are now isolating two new metabolites of vitamin D with which we propose to study intestinal calcium transport. We are proposing under other support to synthesize 3-deoxy-1,25-(OH)₂D₃. This compound will have the 1-hydroxyl group but will be missing the 3-hydroxyl function. We are anxious to learn whether it will stimulate intestinal calcium transport and if not we would like to determine whether it will bind to the receptor proteins and hence provide some insight as to molecular mechanism of discrimination against inactive versus active metabolites. Furthermore, it may be that an antagonist to 1,25-(OH)₂D₃ may be found by testing some of these analogs.

The fourth priority will be continued attempts to understand the mechanism whereby the synthesis of 1,25-(OH)₂D₃ is regulated by parathyroid hormone and plasma inorganic phosphorus. We are now developing systems in which we can get very rapid change-over in the ability of chicks to make 1,25-(OH)₂D₃ or 24,25-(OH)₂D₃. The chick provides an additional advantage in that one can study the levels of enzymatic activity in vitro rather than the appearance of product in blood or tissue. With this system we will attempt to study whether the change-over is actinomycin D sensitive or cycloheximide sensitive to determine whether transcription or translation is involved in the regulation.

We will also continue to study other agents which have been reported to inhibit intestinal calcium transport. For example, corticoid treatment or dilantin and phenobarbital treatment have been reported to reduce intestinal calcium transport. We will examine the question of whether this reduced intestinal calcium transport is related in any way to the synthesis of 1,25-(OH)₂D₃. We will first attempt to reproduce inhibition of intestinal calcium transport by glucocorticoids and then we will try to determine whether 1,25-(OH)₂D₃ can overcome this effect and furthermore we will begin studying in detail vitamin D metabolism by methods common in our laboratory to determine whether these agents affect either the 25-hydroxylation or the 1-hydroxylation systems.

The fifth priority will be given to a study of intestinal phosphate transport reaction in response to 1,25-(OH)₂D₃. We are anxious to learn more in detail about this mechanism to see if it is similar to the 1,25- induced response of intestinal calcium transport. We would like to examine the time course of response, whether it represents change in the brush border surface or the basal-lateral membrane, whether metabolic energy is involved and we would like to prove whether it is active, passive or a facilitated diffusion type of mechanism. We would then begin looking for a phosphate carrier protein or a phosphate dependent hydrolysis of ATP.

Finally, as new systems are developed we will examine the transport of other radionuclides, especially emphasis will be given to potential environmental pollutants such as radium, polonium, beryllium, lead, mercury and cadmium.

We have been maintained on a budget of about \$20-22,000 per year since this project was initiated a number of years ago. The cost of living and prices have changed almost 6% per year since that time. Roughly then our level of support has diminished to almost half of what it was originally. It is getting very difficult to support the activity of even one postdoctoral fellow on this project. We have had to discontinue studies of the vascularly perfused intestine simply because funding levels were too low to permit this. We respectfully request that additional funds be made available to us at least to replace some of the purchasing power lost by the rising cost of living. The suggested budget reflects such an increase.

ENDORSEMENTS

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