In Vitro Cellular Muscle Calcium Metabolism. Characterization of Effects of 1,25-Dihydroxy-Vitamin D_3 and 25-Hydroxy-Vitamin D_3

Ana R. de Boland and Ricardo Boland

Departamento de Biología, Universidad Nacional del Sur, 8000 Bahia Blanca, Argentina

Z. Naturforsch. **40 c**, 102–108 (1985); received September 10/October 24, 1984

1,25-Dihydroxy-vitamin D_3, 25-Hydroxy-vitamin D_3, Cellular Calcium, Muscle Culture, Myoblasts

Cultures of vitamin D-deficient chick soleus muscle and 12 day-old chick embryo myoblasts were used to characterize the effects of 1,25-dihydroxy-vitamin D_3 and 25-hydroxy-vitamin D_3 on muscle cell Ca metabolism. Physiological amounts of both sterols increased the rate and extent of ^{45}Ca uptake by cultures. However, $1,25(OH)_2D_3$ was significantly more effective than $25\,OHD_3$. The greater potency of $1,25(OH)_2D_3$ to increase Ca uptake could be shown after various treatment intervals of cultures and using a wide concentration range of both derivatives. Information about Ca pools affected by vitamin D_3 metabolites was obtained through kinetic analysis of Ca efflux in cultured myoblasts. Cytoplasmic and mitochondria Ca pools were identified on the basis of their half-times of desaturation and by selective inhibition of plasma membrane and mitochondrial Ca transport with $LaCl_3$ and Ruthenium Red, respectively. The data suggests that $1,25(OH)_2D_3$ acts on muscle cellular Ca by increasing Ca efflux and influx through mitochondrial and plasma membranes whereas the predominant effect of $25\,OHD_3$ is to increase Ca influx into mitochondria.

Introduction

Recent investigations have suggested a role of vitamin D_3 in the regulation of intracellular Ca^{2+} levels in skeletal muscle. Studies on the effects *in vivo* of the sterol and derived metabolites have implied $1,25(OH)_2D_3$ [1–3] or $25\,OHD_3$ [4] in Ca^{2+} transport across sarcoplasmic reticulum membranes. An effect of vitamin D_3 on the ability of mitochondria to store calcium has been reported [5]. In addition, more recent research has shown that administration of vitamin D_3 to vitamin D-deficient chicks stimulates ATP-dependent Ca uptake by isolated skeletal muscle sarcolemma vesicles [6]. This evidence does not necessarily imply a direct action of vitamin D_3 and/or its metabolites on muscle Ca metabolism as their effects could be mediated by changes in blood mineral composition or parathyroid hormone levels. However, recent work has indicated a direct involvement of $25\,OHD_3$ and $1,25(OH)_2D_3$ on cellular Ca in cultured chick soleus muscle and myoblasts [7]. At physiological levels, $1,25(OH)_2D_3$ proved to be more effective than 25-OH-D_3 to stimulate ^{45}Ca uptake by these *in vitro* preparations. However, the measurements were made at only one time point and the possibility could not be ruled out that the response of 25-OH-D_3 could have exceed that of 1,25-$(OH)_2$-D_3 at other treatment intervals. In addition, analysis of desaturation kinetics of soleus muscle prelabelled with ^{45}Ca provided further evidence on the effects of both metabolites on muscle Ca fluxes. As these studies were done with intact tissue, where a contribution of interstitial and vascular spaces could be expected, no definitive identification of the physiological correlates of Ca pools obtained from analysis of desaturation curves could be made. A more straightforward interpretation of kinetics of Ca efflux is given by cell culture suspension [8, 9]. The use of specific inhibitors of membrane Ca transport systems [10] may, in addition, contribute to the identification of cell Ca pools. The objective of this work was to further clarify the action of vitamin D_3 metabolites on muscle Ca metabolism. Detailed characterization of variations in Ca uptake by soleus muscle and myoblast preparations in response to *in vitro* treatment with $1,25(OH)_2D_3$ and $25\,OHD_3$ was carried out. Studies on kinetics of Ca desaturation of prelabelled myoblasts were also

Abbreviations: $25\,OHD_3$, 25-hydroxy-vitamin D_3; $1,25(OH)_2D_3$, 1,25-dihydroxy-vitamin D_3; SDS, sodium dodecyl sulfate; R.R., Ruthenium Red.

Reprint requests to Dr. Ricardo Boland.

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performed to obtain information about Ca transport pools modified by the sterols. The effects of Ruthenium Red and $LaCl_3$ on myoblast Ca fluxes were evaluated to identify these pools.

Materials and Methods

Cultures

Isolated myoblasts from 12 day-old chicken embryo breast muscle and intact soleus muscles from vitamin D-deficient chicks were obtained as previousy described [7].

Soleus muscles were treated with 25 OHD_3 and $1,25(OH)_2D_3$ for various times (1–8 h) in a chemical defined medium [11] containing 1 mM Ca^{2+} and supplemented with 10% vitamin D-deficient chick serum. The sterols were used dissolved in ethanol. Solvent alone was added to control muscles. Incubations were performed in a Dubnoff Incu-Shaker at 37 °C under air-CO_2 (95% : 5%). To culture muscle cells, the suspension of myoblasts were freed of fibroblasts by preplating on gelatin coated Petri dishes and brought to a concentration of 5×10^5 cells/ml in Eagle's Minimum Essential Medium containing 1 mM Ca^{2+}, 10% serum from vitamin D-deficient chicks, 10% chick embryo extract and 1% antibiotic-antimycotic solution [12]. Three milliliters of this suspension were plated on a 60 mm Petri dish and incubated at 37 °C in a water-jacketed incubator (Forma Scientific, USA) under a constant flow of 95% air-5% CO_2 saturated with water. The medium was replaced after 24 h incubation, lowering chick embryo extract to 2%. Vitamin D_3 metabolites were added at this time and the plates were cultured for up 24 h. Viability of the cells from control and treated preparations was ensured by measurement of trypan blue exclusion.

Levels of 25 OHD_3 (20 ng/ml) and $1,25(OH)_2D_3$ (0.05 ng/ml) used to treat both soleus and myoblast cultures were close to those normally found in chick serum [13].

Calcium uptake and efflux

Calcium uptake by intact soleus muscles was measured using a 60 min incubation at 37 °C in Krebs-Henseleit-bicarbonate saline solution containing 1 mM $^{45}CaCl_2$ (0.2 μCi/ml) as described before [7]. To evaluate Ca uptake by suspended cells, the myoblast preparations were preincubated at 37 °C with Krebs-Henseleit-glucose solution [14] for 60 min to achieve steady-state conditions. $^{45}CaCl_2$ (1 mM, 0.5 μCi/ml) was then added to the medium and the cells were incubated for various time intervals (5–90 min). A 1 ml aliquot of cell suspension was added to 40 ml of ice-cold Krebs-Henseleit solution contained in a centrifuge tube and centrifuged for 45 s at 3500 rpm. The supernatant was discarded and the cell pellet was solubilized by heating for 15 min at 55 °C in 0.1% SDS. Aliquots were taken for measurement of radioactivity in a Beckman liquid scintillation spectrometer and protein determination by the Lowry procedure [15].

Calcium efflux studies were performed with myoblasts cultured in 250 μCa^{2+} to avoid their clumping and fusion. A similar design as described for kidney cells [8] was used. The cells were prelabelled with $^{45}CaCl_2$ (1 μCi/ml) at 37 °C as for uptake measurements. After labelling the cell suspension was centrifuged for 30 s at 2000 rpm, the radioactive supernatant decanted from the tube and the cells were again rapidly washed and resuspended in fresh non-radioactive Krebs-Henseleit solution. The cells were divided into two tubes and placed in a water bath at 37 °C under shaking to perform isotope desaturation. The medium was replaced at 5, 10, 20, 30, 60, 90, 120, 150 and 180 min. After each centrifugation an aliquot of the medium was taken for radioactivity measurements. At the end of the desaturation period the cells were dissolved by heating in 0.1% SDS to determine ^{45}Ca radioactivity and protein content.

To evaluate the effects of Ruthenium Red and $LaCl_3$, these compounds were included in the medium at concentrations of 100 μM and 200 μM, respectively, during both the prelabelling and desaturation periods.

Material

Purified samples of chemically synthetized $1,25(OH)_2D_3$ and 25 OHD_3 were provided by Dr. M. Uskokovic (Hoffman-LaRoche Co., New Jersey, USA) and Dr. A. J. Campbell (The Upjohn Co., Michigan, USA) respectively. Ruthenium Red was obtained from Sigma Chemical Co. (Saint Louis, USA). All other reagents were of analytical grade.

Results

The time course of Ca uptake by myoblast suspensions cultured in the absence or presence of physiological levels of vitamin D_3 metabolites was characterized, the results shown in Fig. 1. Uptake was linear for at least 20 min and maximum values were attained after 30 min incubation. Values at zero time are likely to represent the non-specific adsorption of labelled Ca to the cells. A clear stimulation of the "initial rate" (values at 5 min) and total Ca uptake capacity (values at 30 min) of the cells by $1,25(OH)_2D_3$ and $25OHD_3$ could be seen. The effects of $1,25(OH)_2D_3$, however, were significantly higher than those produced by $25OHD_3$. Calcium uptake by soleus muscle from vitamin D-deficient chicks was linear up to 60 min. Similarly as observed with myoblast preparations, $1,25(OH)_2D_3$ proved to be more effective than $25OHD_3$ to stimulate Ca uptake (data not shown).

The time courses of the $1,25(OH)_2D_3$ and $25OHD_3$ Ca uptake responses were characterized in intact soleus muscle and myoblast cell cultures. Ca uptake sharply increased after 1 h treatment of soleus muscles with $1,25(OH)_2D_3$, as shown in Fig. 2. The response to the metabolite continued to increase more gradually for up to 5 h, when a maximum response was observed. Then Ca uptake markedly decreased at 8 and 10 h. In the case of $25OHD_3$, a significant increase in the response could be seen after 2 h treatment. Calcium uptake gradually declined, then, between 2 h and 10 h. The stimulation in Ca uptake induced by this metabolite was smaller than that elicited by $1,25(OH)_2D_3$. The effects of metabolites on Ca uptake by myoblast cultures were clearly evident after a longer period of treatment. Maximal stimulation of calcium uptake was obtained with $25OHD_3$ after 8 h of treatment. ^{45}Ca labeling remained essentially constant during subsequent culture of the cells in presence of the metabolite. The response to $1,25(OH)_2D_3$ was significantly higher and continued to increase linearly for 24 h (Fig. 3).

Dose-response relationships were studied in soleus preparations cultured for 5 h in the presence of a wide concentration range of vitamin D_3 metabolites ($1,25(OH)_2D_3$: 0.01–1.0 ng/ml; $25OHD_3$: 1.0–100 ng/ml). The most effective concentrations of $1,25(OH)_2D_3$ and $25OHD_3$ were 0.05 ng/ml (48% increase over control) and 20 ng/ml (20% increase over control), respectively. In myoblast cultures treated with $1,25(OH)_2D_3$ during 24 h, Ca uptake responses as high as 100% over controls could be observed by increasing concentrations from 0.05 to 0.15 ng/ml. However, no further significant increase

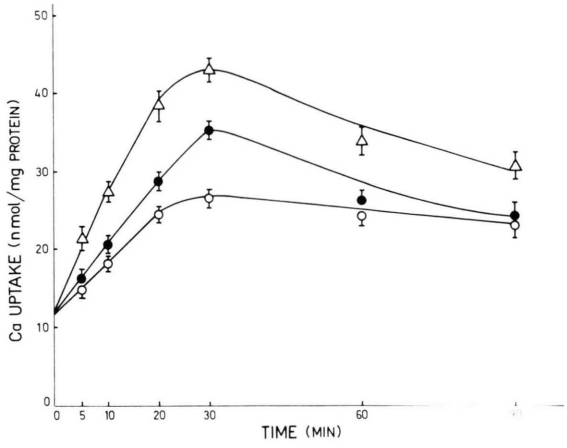

Fig. 1. Effects of vitamin D_3 metabolites on the time course of Ca uptake by cultured myoblasts. $1,25(OH)_2D_3$ (0.05 ng/ml) and $25OHD_3$ (20 ng/ml) were added to 24 h cultures. Samples were cultured for another 24 h in presence of metabolites and were then incubated for increasing times in buffer containing ^{45}Ca as described under Material and Methods. (○-----○) control; (●-----●) $25OHD_3$; (△-----△) $1,25(OH)_2D_3$. Values are means ± SD; $n = 4$.

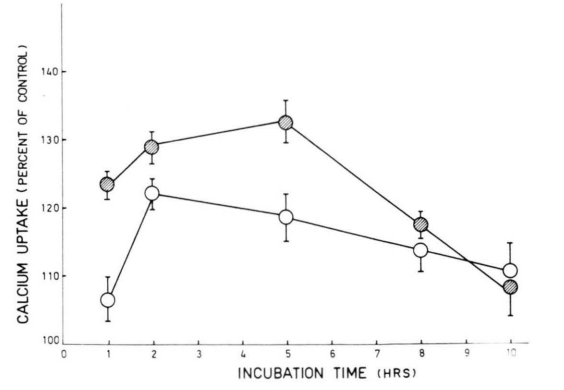

Fig. 2. The time course of changes in Ca uptake in cultured soleus muscles induced by vitamin D_3 metabolites. Soleus muscles from vitamin D-deficient chicks were cultured for various times with $1,25(OH)_2D_3$ (0.05 ng/ml) and $25OHD_3$ (20 ng/ml). Calcium uptake was measured in Krebs-Henseleit solution containing ^{45}Ca as described under Material and Methods. (○----○) $25OHD_3$; (●-----●) $1,25(OH)_2D_3$. Values are means ± SD; $n = 4$.

in $25OHD_3$ responses could be seen at levels higher than 20 ng/ml (data not given).

Higher ^{45}Ca labelling of cells treated with $1,25(OH)_2D_3$ and $25OHD_3$ might be associated to effects of the sterols on Ca fluxes across plasma and/or intracellular membranes. To obtain information about the Ca pools affected by vitamin D_3 metabolites the kinetics of Ca efflux of myoblast suspensions prelabelled with ^{45}Ca was studied. Efflux curves obtained with myoblasts were similar to those previously described for other cell types [8, 9]. A representative experiment is shown in Fig. 4. Their graphical analysis revealed 3 rates of efflux, indicating the presence of a "very fast"-, a "fast"- and a "slow"-exchangeable Ca pool. These pools were characterized by half-times of desaturation of 1–2 min, 10–15 min and 100–120 min, respectively. Kinetic parameters of compartments were calculated using equations which may be applied to the analysis of Ca desaturation curves even if isotopic equilibrium has not been reached during prelabelling with tracer [16]. In Table I, values of the rate constants of efflux and size of the "fast"- and "slow"-turnover pools are given. Parameters of the "very fast" component of efflux were not estimate since the technique of isotope desaturation is not fast enough to measure it with accuracy. $1,25(OH)_2D_3$ caused a significant increase in the rate of efflux from the "slow"-turnover Ca pool (k_{32}) and in the size of the "fast"-turnover Ca pool (S_2). Less significant increases were obobserved in K_{23} and K_{20} by addition of the metabolite to the cells. On the other hand, the main effect of $25OHD_3$ consisted in a marked increase in the rate constant of flux from the "fast"- to the "slow"-Ca pool (k_{23}). A large increase in the size of the "slow"-exchangeable compartment (S_3) was also observed whereas S_2 was augmented less pronouncedly. The "very fast"-, "fast"- and "slow"-exchangeable Ca pools have been associated in other cells to the extracellular glycocalyx, cytoplasm and mitochondria, respectively [8, 9]. To further characterize the nature of the pools in muscle cells affected by vitamin D_3 metabolites, the effects of ruthenium red and $LaCl_3$ on Ca fluxes were characterized. These compounds have been reported to specifically inhibit *in vivo* Ca transport processes located at mitochondria and plasma membrane, respectively [10]. The effects of both compounds on kinetic parameters of Ca efflux in cultured cells are described in Table II. Ruthenium red decreased k_{23}, S_2 and S_3. Main changes of cells exposed to 200 µM $LaCl_3$ were a 3.5-fold decrease of k_{20} and an increase of S_2 (20-fold) and S_3 (16-fold).

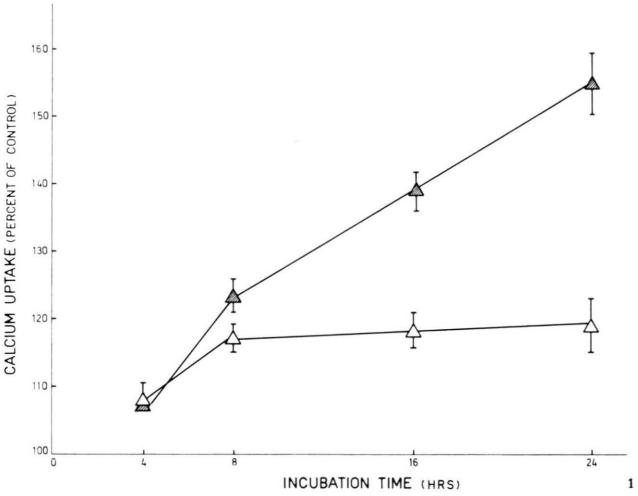

Fig. 3. The time course of changes in Ca uptake in cultured myoblasts induced by vitamin D_3 metabolites. 24-h myoblast cultures were incubated for various times with $1,25(OH)_2D_3$ (0.05 ng/ml) and $25OHD_3$ (20 ng/ml). Calcium uptake was measured in buffer containing ^{45}Ca as described under Material and Methods. (△----△) $25OHD_3$; (▲-----▲) $1,25(OH)_2D_3$. Values are means ± SD; $n = 4$.

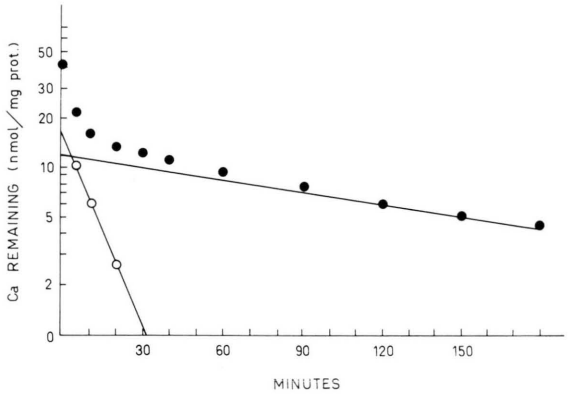

Fig. 4. Typical ^{45}Ca efflux curve obtained in cultured myoblasts in Krebs-Henseleit buffer containing 1.0 mM calcium. Efflux measurements were performed as described under Material and Methods. Calcium retained in the cells (nmol/mg prot) is plotted versus time. Two different phases were evaluated by graphical analysis [8, 9]. (●-----●) desaturation of the total cell culture; (○-----○) calculated desaturation of the "fast"-turnover Ca compartment.

Table I. The effect of $1,25(OH)_2D_3$ and $25OHD_3$ on Ca pool sizes and rate constants of fluxes in cultured muscle cells. Chick embryo myoblasts were cultured in the presence of 250 μM Ca as previously described [7]. Cells were treated with $1,25(OH)_2D_3$ (0.05 ng/ml) and $25OHD_3$ (20 ng/ml) during 24 h. The suspension was labelled with ^{45}Ca for 1 h and desaturation was performed during an additional 3 h period. K, min^{-1}, and S, nmol × mg prot.$^{-1}$, were calculated as described by Uchikawa and Borle [16]. K_{20}, K_{23} and K_{32} are the rate constants of fluxes from the "fast"-turnover pool to the medium (k_{20}), from the "fast"-turnover Ca pool to the "slow"-turnover Ca pool (K_{23}), and from the "slow"-turnover Ca pool to the "fast"-turnover Ca pool (K_{32}). S_2 and S_3 are the pool sizes of the "fast"- and "slow"-turnover Ca pools, respectively. Values were calculated from 4 separate experiments and represent means ± S.D.

	$K_{20} \times 10^2$	$K_{23} \times 10^3$	$K_{32} \times 10^3$	S_2	S_3
Control	2.99 ± 0.14	8.28 ± 0.41	3.0 ± 0.12	29.6 ± 1.48	80.4 ± 4.02
$1,25(OH)_2D_3$	4.06 ± 0.26[a]	9.50 ± 0.53[a]	5.6 ± 0.28[b]	43.2 ± 2.20[b]	72.5 ± 3.62
$25OHD_3$	3.05 ± 0.18	16.61 ± 1.13[b]	3.3 ± 0.16	37.3 ± 2.36[a]	188.1 ± 12.64[b]

[a] $p < 0.01$; [b] $p < 0.005$.

Table II. The effects of Ruthenium Red and $LaCl_3$ on Ca-efflux parameters of cultured myoblasts. Experimental conditions and calculation of kinetic parameters were as those described for Table I. Ruthenium Red (100 μM) and $LaCl_3$ (200 μM) were present in the medium during ^{45}Ca labelling of the cells and the subsequent desaturation period. Values are means of 4 separate experiments ± SD.

	$K_{20} \times 10^2$	$K_{23} \times 10^3$	$K_{32} \times 10^3$	S_2	S_3
Control	2.99 ± 0.14	8.30 ± 0.41	3.02 ± 0.12	29.57 ± 1.38	80.35 ± 4.01
R.R.	3.03 ± 0.23	5.60 ± 0.38	3.60 ± 0.14	17.78 ± 0.78	27.59 ± 1.25
$LaCl_3$	0.87 ± 0.04	7.01 ± 0.25	2.69 ± 0.10	517.26 ± 29.86	1318.7 ± 65.93

Discussion

Characterization of the effects of $1,25(OH)_2D_3$ and $25OHD_3$ on ^{45}Ca uptake by cultured soleus muscle and myoblasts and Ca efflux by prelabelled myoblasts showed distinguishing features about the biological activity and mode of action of both metabolites on muscle Ca metabolism. $1,25(OH)_2D_3$ proved to be more effective than $25OHD_3$ to increase the initial rate and extent of ^{45}Ca labelling of myoblast (Fig. 1) and soleus cultures when added at physiological levels. The initial rate of Ca influx presumably represents Ca transport across the muscle cell plasma membrane. The time course of the $1,25(OH)_2D_3$ and $25OHD_3$ responses showed greater effectiveness of $1,25(OH)_2D_3$ to increase ^{45}Ca labelling of intact muscle and cell cultures at all treatment intervals. No major qualitative differences in temporal profiles of Ca uptake by soleus preparations were observed between both metabolites. A more sustained increase in Ca uptake by myoblasts could be seen with $1,25(OH)_2D_3$ than with $25OHD_3$. This may explain better growth and differentiation of cultures treated with $1,25(OH)_2D_3$ than with $25OHD_3$ observed previously [7] as Ca^{2+} promotes muscle cell fusion [12, 17]. Evaluation of the effects of both metabolites on ^{45}Ca uptake by muscle cultures employing a wide concentration range confirmed the greater potency of $1,25(OH)_2D_3$.

"Fast" and "slow"-exchangeable compartments were identified by the analysis of ^{45}Ca desaturation curves of isolated myoblasts. It may be safely assumed that these compartments represent cytoplasmic and mitochondrial Ca pools, respectively. Their half-times of desaturation were similar to those reported for several other cell types, where experimental evidence about their intracellular location has been given [8, 9, 18]. Characterization of the effects of $LaCl_3$ and ruthenium red provided additional criteria for their identification. It was possible to expand the size of the "fast" pool 20-fold and to markedly reduce its rate constant of efflux k_{20} in the presence of 200 μM $LaCl_3$. These changes may be explained by the selective inhibitory action of La^{3+} in intact cells on the plasma membrane Ca efflux pump [10, 19] and indicate that this pool represents cytoplasmic calcium. The size of the "slow-exchangeable" compartment was decreased 3-fold by the addition of Ruthenium Red to muscle cell cultures. K_{23} was also diminished. The modifications are consistent with the fact that R.R. is an

inhibitor of mitochondrial Ca^{2+} transport *in vivo* [10, 19]. The contribution of sarcoplasmic reticulum, which represents a major mechanism of accumulation of intracellular calcium in differentiated muscle, to the "slow" pool should not be relevant as it is only scarcely present in cultured myoblasts of this age [20].

The data from desaturation experiments suggest that $1,25(OH)_2D_3$ stimulates bidirectional Ca fluxes across mitochondrial and plasma membranes in muscle cells. A distinctive modification caused by the metabolite was an increase in the rate constant of efflux of Ca from mitochondria (K_{32}). The metabolite also increased the rate of Ca influx from cytoplasm into mitochondria (K_{23}). In addition, the rate constant of Ca^{2+} efflux from cytoplasm to the external medium (K_{20}) was augmented by pretreatment of cells with $1,25(OH)_2D_3$. However, an increase in the size of the cytoplasmic Ca pool (S_2) was observed. This may be explained by an increased Ca influx across the cell membrane as suggested by the effects of $1,25(OH)_2D_3$ on the "initial rate" of Ca uptake (Fig. 1). In bone cell cultures it has also been shown that $1,25(OH)_2D_3$ stimulates Ca fluxes in a "slow"-exchangeable compartment and the initial rate of Ca influx into the cells [18]. The pronounced increase in K_{23} and S_3 observed in myoblasts cultured in the presence of $25\,OHD_3$ implies that this metabolite acts primarily stimulating mitochondrial Ca uptake.

The effects of $1,25(OH)_2D_3$ on both Ca influx and efflux from myoblasts suggests that the metabolite causes a greater increase in muscle cell Ca turnover than $25\,OHD_3$ which only stimulates Ca influx. This may explain higher ^{45}Ca labelling of soleus and myoblast preparations treated with $1,25(OH)_2D_3$ than with $25\,OHD_3$.

Previous studies of kinetics of Ca efflux in chick soleus muscle preparations suggested similar differential characteristics of the effects of vitamin D_3 metabolites on muscle cellular calcium [7]. This close agreement between the results obtained with embryonic muscle cells and the more differentiated soleus muscle indicates that myoblasts cultures may constitute a valid model to characterize the effects and mechanism of action of vitamin D_3 metabolites on skeletal muscle Ca metabolism.

The results obtained by tissue culture are in agreement with previous observations on the effects *in vivo* of vitamin D_3 and related metabolites on muscle Ca fluxes. Thus, Bauman *et al.* [21] have shown that vitamin D depletion of chicks leads to Ca accumulation in muscle tissue. Administration of $1,25(OH)_2D_3$ reversed this change. Increased Ca efflux from mitochondria and cytoplasm in muscle cells treated with $1,25(OH)_2D_3$ observed in this study may provide a mechanism of $1,25(OH)_2D_3$ action on Ca mobilization from skeletal muscle of rachitic animals. Moreover, in agreement with these observations it has been reported that administration of vitamin D_3 to vitamin D-deficient chicks stimulates the Ca-dependent ATPase of skeletal muscle sarcolemma [6]. In addition, a marked stimulation of Ca efflux from muscle mitochondria preloaded *in vitro* with ^{45}Ca has been observed upon treatment of vitamin D-deficient chicks with vitamin D_3 [5].

There is scarce information about possible mechanisms by which the effects of $25\,OHD_3$ and $1,25(OH)_2D_3$ are mediated. The action of the sterols on mitochondrial Ca fluxes may involve the synthesis of specific proteins. It has been recently reported that vitamin D_3 affects *in vivo* the composition and [^{3}H]leucine labelling of proteins of chick muscle mitochondrial membranes [22, 23]. These changes may be connected to $25\,OHD_3$ as only a receptor for this metabolite has been found in rat skeletal muscle [11]. However, in primary cultures of bone cells it has been shown that the addition of protein synthesis inhibitors abolishes the increase in Ca efflux from mitochondria induced by $1,25(OH)_2D_3$ [18]. Similar studies with muscle cells are in progress in our laboratory. The pronounced increase in mitochondrial Ca influx kinetic parameters caused by $25\,OHD_3$ may, in addition, be related to its stimulatory effect on muscle PO_4 uptake [11], as Ca accumulation by mitochondria is affected by cytosolic PO_4 levels [9]. The action of $1,25(OH)_2D_3$ on Ca fluxes across muscle cell plasma membrane may not be mediated by protein synthesis. Vitamin D_3 induces *in vivo* significant increases in phosphatidylcholine and sphyngomyelin contents of skeletal muscle sarcolemmal membranes [6]. Similar changes are produced by $1,25(OH)_2D_3$ in cultured myoblasts (Boland, A. R. de and Boland, R.; unpublished data). It has been reported that $1,25(OH)_2D_3$ affects the lipid composition of intestinal plasma cell membranes by a mechanism independent of protein synthesis. These changes have been correlated to the effects of the metabolite on Ca transport [24, 25].

Acknowledgements

This research was supported by grants from the Comision de Investigaciones Cientificas de la Provincia de Buenos Aires and the Consejo Nacional de Investigaciones Científicas y Tecnicas, Argentina.

[1] C. Matthews, K. W. Heimberg, E. Ritz, B. Agostini, J. Fritzsche, and W. Hasselbach, Kidney Int. **11,** 227 (1977).
[2] R. L. Boland, C. Matthews, A. R. de Boland, E. Ritz, and W. Hasselbach, Calcif. Tiss. Int. **35,** 195 (1983).
[3] R. L. Boland, A. R. de Boland, E. Ritz, and W. Hasselbach, Calcif. Tiss. Int. **35,** 190 (1983).
[4] J. J. Pointon, M. J. O. Francis, and R. Smith, Clinical Science **57,** 257 (1979).
[5] D. Pleasure, B. Wyszynski, A. Sumner, D. Schotland, B. Feldman, H. Nugent, H. Hitz, and D. B. P. Goodman, J. Clin. Invest. **64,** 1157 (1979).
[6] A. R. de Boland, S. Gallego, and R. Boland, Biochim. Biophys. Acta **733,** 264 (1983).
[7] D. L. Giuliani and R. L. Boland, Calcif. Tiss. Int. **36,** 200 (1984).
[8] A. B. Borle, Methods in Enzymology **39,** 513 (1975).
[9] A. B. Borle, Membrane Biol. **10,** 45 (1972).
[10] A. M. Dani, A. Cittadini, G. Calviello, G. Festuccia, and T. Terranova, Mol. Cell. Biochem. **22,** 139 (1978).
[11] S. J. Birge and J. G. Haddad, J. Clin. Inv. **56,** 1100 (1975).
[12] M. C. O'Neill and F. E. Stockdale, J. Cell Biol. **52,** 52 (1972).
[13] R. L. Horst, T. A. Reinhardt, E. T. Littledike, and J. L. Napoli, in: Vitamin D, Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism (A. W. Norman et al., eds.), pp. 757, Walter de Gruyter & Co., Berlin 1982.
[14] J. Paul, Cell and tissue culture, Churchill Livingstone, Great Britain 1975.
[15] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. **193,** 265 (1951).
[16] T. Uchikawa and A. B. Borle, Am. J. Physiol. **234,** R29 (1978).
[17] J. J. Van der Bosch, C. H. R. Schudt, and D. Pette, Expt. Cell Res. **82,** 433 (1973).
[18] Y. Eilam, N. Szydel, and A. Harell, Mol. Cell Endocrinol. **19,** 263 (1980).
[19] A. Cittadini, A. Scarpa, and B. Chance, Biochim. Biophys. Acta **291,** 246 (1973).
[20] D. A. Fischman, Curr. Top. Dev. Biol. **5,** 235 (1970).
[21] V. K. Bauman, M. Y. Valinietse, and D. A. Babarykin, Arch. Biochem. Biophys. **231,** 211 (1984).
[22] A. R. de Boland, L. Albornoz, and R. Boland, Calcif. Tiss. Int. **35,** 798 (1983).
[23] A. R. de Boland and R. Boland, Z. Naturforsch. **39c,** 1015 (1984).
[24] T. Matsumoto, O. Fontaine, and H. Rasmussen, J. Biol. Chem. **256,** 3354 (1981).
[25] H. Rasmussen, O. Fontaine, and T. Matsumoto, Annals N.Y. Acad. Sc. **341,** 518 (1981).