Published in final edited form as:

Steroids. 2010 ; 75(8-9): 555–559. doi:10.1016/j.steroids.2009.12.001.

Membrane Localization, Caveolin-3 Association and Rapid Actions of Vitamin D Receptor in Cardiac Myocytes

Guisheng Zhao and Robert U. Simpson

Department of Pharmacology, University of Michigan, School of Medicine, Ann Arbor, Michigan, USA

Abstract

The active form of vitamin D, 1 α , 25-Dihydroxyvitamin D₃ (1, 25(OH)₂D₃), mediates both genomic and rapid non-genomic actions in heart cells. We have previously shown that the vitamin D receptor (VDR) is located in the t-tubular structure of cardiomyocytes. Here we show that VDR specifically interacts with Caveolin-3 in the t-tubules and sarcolemma of adult rat cardiac myocytes. Co-Immunoprecipitation studies using VDR antibodies revealed that Caveolin-3 specifically co-precipitates with the VDR and similarly the VDR is co-precipitated with Caveolin-3 antibody. Confocal immunofluorescence microscopy analysis also showed co-localization of VDR and Caveolin-3 in t-tubules and sarcolemma. The non-genomic effects of the functional VDR were studied in electrically stimulated myocytes isolated from adult rat hearts. Sarcomere shortening and re-lengthening were measured in 1, 25 (OH)₂D₃ treated cardiac myocytes. A 1nM treatment decreased peak shortening within minutes, suggesting a rapid effect through the membrane-bound VDR. This novel finding of the interaction between VDR and Caveolin-3 is fundamentally important in understanding 1, 25(OH)₂D₃ signal transduction in heart cells and provides further evidence that VDR plays a role in regulation of heart structure and function.

Introduction

1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃) exerts its effects through the vitamin D receptor (VDR), a ligand-activated transcription factor expressed in a wide array of tissues and cell types including heart [1,2]. Vitamin D₃ deficiency and reduced levels of the active vitamin D metabolite 1,25(OH)₂D₃ have been associated with the etiology and pathogenesis of congestive heart failure (CHF) [3]. Studies from our lab demonstrated that vitamin D₃ deficiency alters myocardial function, morphology and extracellular matrix (ECM) [2,4,5]. We have also shown that ablation of the VDR signaling system in VDR knockout mice results in profound changes in heart structure and function [6].

Though traditionally the VDR has been characterized as a nuclear steroid receptor that modulates gene transcription upon binding of its ligand, 1,25(OH)₂D₃ has also been shown to exert rapid, non-genomic responses involving stimulation of trans-membrane signal transduction pathways through putative membrane associated receptors [7-10]. The VDR has been found in isolated membrane fractions of both chick intestinal cells, and chick embryonic

© 2009 Elsevier Inc. All rights reserved.

*Address correspondence to: Robert U. Simpson Department of Pharmacology, University of Michigan Medical School 1150 West Medical Center Drive MSRB III, Room 1301. Ann Arbor, MI 48109. Phone: (734) 763-3255 Fax: (734) 763-4450 robsim@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

skeletal muscle cells [11]. Our lab has reported that in adult rat and mouse cardiomyocytes VDR is located in the t-tubular membrane structures [12], yet the specific interactions between VDR and t-tubular membrane proteins remain unclear.

The t-tubules of mammalian cardiac ventricular myocytes are invaginations of the plasma membrane. The development of t-tubules appears to depend on proteins and lipids and shows properties that are similar to the development of caveolae, which requires cholesterol and Caveolin-3 [13]. Caveolins are the principle structural proteins that are both necessary and sufficient for the formation of caveolae membrane domains. These proteins function both in protein trafficking and signal transduction, as well as in cholesterol homeostasis [14,15]. Caveolae-associated VDR in the plasma membranes has been found in other tissues, however the interaction between VDR and caveolae and its function in cardiomyocytes has not been demonstrated. In this report, we show that the VDR localizes to the t-tubule and sarcolemma of rat cardiomyocytes and co-immunoprecipitates with the membrane protein Caveolin-3. Moreover, we report that $1,25(\text{OH})_2\text{D}_3$ rapidly (within minutes) modulates the contraction of isolated cardiomyocytes.

Materials and Methods

Animals

All procedures involving animals were executed in accordance with the guidelines of the University Committee on the Use and Care of Animals (UCUCA) of the University of Michigan. Three to six-month-old male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were used in this study. These rats were housed in the University of Michigan Laboratory Animal Facility in standard cages with a 12-h light, 12-h dark cycle. Rats were fed standard rodent chow and water.

Isolation of rat ventricular myocytes

Ventricular myocytes were isolated from rat hearts as previously described [16]{Green, 2006 #1}. Male Sprague-Dawley rats (250–300g) were pre-treated with 0.01 U/kg heparin IP followed 10 minutes later by a lethal dose of pentobarbital. The heart was quickly removed and mounted on a Langendorff apparatus, and retrogradely perfused with Krebs-buffered solution (KREBS) containing (in mmol/l): 118 NaCl, 4.8 KCl, 1.2 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 25 HEPES, 1.2 KH_2PO_4 , and 11 glucose (pH set to 7.4 using HCl). When the coronary circulation had cleared of blood, perfusion was continued with Ca^{2+} -free KREBS isolation solution for 3 min, followed by perfusion for a further 30 min with Ca^{2+} -free KREBS isolation solution containing 6750 U of collagenase type 2 (Worthington Biochemical Corporation, Lakewood, NJ) and 12 mg of hyaluronidase (Sigma, St. Louis, MO). Calcium concentration was gradually increased to 0.75 mM during the digestion. The ventricles were then excised, minced, and gently shaken at 37°C in the collagenase-containing solution. Ventricular cells were collected from this solution at 5 min intervals and re-suspended in KREBS containing 1% BSA and 1.75 mM CaCl_2 . Cells were then plated onto laminin-coated coverslips in DMEM supplemented with 50 U/ml penicillin, 50 $\mu\text{g}/\text{ml}$ streptomycin (Pen/Strep, Life Technologies), and 5% serum. Cells were incubated at 37°C for 2 hrs in 2% CO_2 .

Isolated cardiac ventricular myocytes contraction studies

Cells were plated as above. After 2-hr incubation the plating media was changed to media 199 supplemented with pen/strep (M199), 10 mM HEPES, 0.2 mg/ml bovine serum albumin, and 10 mM glutathione (M199+). Individual coverslip was transferred to a temperature-controlled stimulation chamber containing platinum electrodes mounted to the sides of each well and a glass bottom mounted on a Nikon microscope. Myocytes were electrically stimulated (Myopacer, 2.5 ms pulse, 0.5 Hz, 4 V) and the chamber was perfused with M199+. Sarcomere

shortening was detected using a video-based detection system (Ionoptix, Milton, MA) on intact myocytes. Recordings were made before and 1, 2.5, 5, 10, and 15, 30 min after addition of calcitriol (10^{-9} M; Sigma). Ten twitches per myocyte were collected for each sample. The shortening transient was indistinguishable over this same time interval in non-stimulated myocytes. Signal averaged data were analyzed to determine resting sarcomere length, peak shortening normalized for resting sarcomere length (% peak height), time to peak shortening (TTP), time to 25, 50, and 75% relaxation (TTR25, TTR50, and TTR75, respectively), and maximum normalized shortening and relaxation velocities ($+dI/dt_{max}$, $-dI/dt_{max}$, respectively).

Immunocytochemistry

Immunofluorescent staining of cells was carried out in a method similar to that previously described [17][Nibbelink, 2006 #29]. Briefly, laminin-coated coverslip-plated cells were washed in PBS and then fixed in 10% neutral buffered formalin (Sigma, St Louis, MO) for 10 min, rinsed in PBS, and blocked in 10% Donkey serum in PBS-0.05% Triton-X 100 (PBS-T). Cells were incubated with rabbit VDR antibody (sc-1008; 1:100; Santa Cruz, CA) and mouse caveolin-3 antibody (sc-5310; 1:200; Santa Cruz) diluted in 5% Donkey serum/PBS-T at 4°C overnight. After incubation, cells were rinsed in PBS and then incubated with donkey anti-rabbit Alexa488, donkey anti-mouse Alexa594 (Invitrogen, Carlsbad, CA) secondary antibody diluted in 5% Donkey serum in PBS-T for 1 hour at room temperature. Cells were then washed in PBS, and mounted in ProLong Gold (Invitrogen). Slides were analyzed with Olympus FV-500 imaging software on an Olympus iX 81 confocal microscope and finalized using Adobe Photoshop (Adobe Systems Inc).

Co-Immunoprecipitation of Cav-3 and VDR

Polyclonal VDR antibody (100 ug; sc-1008 from Santa Cruz), monoclonal VDR antibody (100 ug, MA1-710 from Affinity Bioreagents), or monoclonal caveolin-3 antibody (100 ug, sc-5310 from Santa Cruz) were covalently immobilized overnight at room temperature with gentle end-over-end mixing to 50 ul of Antibody Coupling Gel (AminoLink Plus Gel, Pierce, Rockford, IL) respectively into a Handee Spin Cup column as recommended by the manufacturer (Pierce, Rockford, IL). Isolated rat cardiomyocytes and intestinal mucosa were prepared as previously described (Tishkoff et. al., 2008, Endocrinology). Both isolated cardiomyocytes and intestinal mucosa were incubated with 10 volumes (v/v) of Pierce M-PER® extraction reagent for 10 min at room temperature with rocking, then sonicated on ice 6 times at 50% power, 12 bursts each time. After centrifuging at 27000 g for 15 min to get the protein supernatant, 600 ug of cardiomyocyte and intestinal lysate were incubated with the VDR gel resin or caveolin-3 gel resin and “quenched control” resin for 2 hr at room temperature, then washed and eluted off in 50 ul fractions 4 times as recommended by the manufacturer (Pierce). 20 ul of eluted samples from the first 3 fractions was subjected to Western Blot analysis by running on a 12.5% Criterion SDS-PAGE Tris HCl gel (Bio-Rad Laboratories, Hercules, CA) and transferred to an Immobilon-P polyvinyl difluoride membrane (Millipore, Bedford, MA). Membrane was blocked for 1 hr in 5% nonfat dry milk and then incubated with primary VDR antibody (sc-1008, rabbit polyclonal, 1:200, Santa Cruz) diluted in 5% milk/Tris-buffered saline containing 0.05% Tween 20 (TBST) for 1 hr at room temperature. After four 5-min washes with TBST, membrane was incubated with a secondary antibody conjugated with horseradish peroxidase. The blot was repetitively stripped and reprobed with other primary antibodies: a 1:200 dilution of the caveolin-3 antibody (sc-5310 from Santa Cruz). Secondary antibodies used were: a 1:1000 dilution of NA934 anti-rabbit for VDR (GE Healthcare), and a 1:1000 dilution of NA931 anti-mouse for Cav-3 (GE Healthcare).

Statistical Analysis

In all experiments significant differences between data sets was determined using the unpaired student's t-test. A two-sided P value of <0.05 was considered significant. Data are presented as means $\pm$ SE unless otherwise stated.

Results

Since caveolae-associated VDR in the plasma membrane has been observed in other tissues, we examined whether VDR is also associated with caveolae in cardiomyocytes. Immunocytochemistry and confocal microscopy was used to identify the co-localization of VDR with the heart-specific caveolin protein, Caveolin-3 (Figure 1). Consistent with our previous findings (12), a strong immuno-staining of VDR was seen in isolated adult rat ventricular cells in a repeating pattern of transverse lines throughout the cell (figure 1a, d). Caveolin-3 is a major component of cardiac caveolae complex. Double labeling with VDR and Caveolin-3 antibodies showed similar patterns of immunoreactivity, suggesting that these two proteins are closely associated (figure 1). High resolution imaging of all examined cells similarly showed a clear pattern of association between VDR and caveolin-3 in the t-tubules and sarcolemma. These data demonstrated that in ventricular cardiac myocytes, membranous VDR is closely associated with Caveolin-3 and thus the caveolae complex in cardiomyocyte membranes.

To further study the interactions between VDR and rat cardiomyocyte t-tubule and sarcolemma proteins, polyclonal (SC-1008, Santa Cruz), and monoclonal (MA1-710, Affinity Bioreagent) VDR antibodies were immobilized to antibody coupling gel (AminoLink Plus gel, Pierce) respectively, to precipitate VDR-associated proteins. The eluted samples were analyzed on SDS-PAGE gel and transferred to PVDF membrane. Western blot analyses were performed. Figure 2A shows that the heart specific caveolin, Caveolin-3, is co-precipitated with VDR. Additionally, a Caveolin-3 Antibody (PA1-066, Affinity Bioreagent) immobilized to coupling gel similarly brought down the VDR (figure 2B), supporting a conclusion that the interaction between VDR and Caveolin-3 is specific.

To examine the function of the membrane associated VDR in cardiac myocytes, contraction parameters of individual intact cardiomyocytes isolated from adult rat heart were studied by measuring sarcomere shortening in response to electrical stimulation before and after 1,25(OH)₂D₃ incubation. The Ionoptic imaging system was utilized as described in the methods section. Our results demonstrate a direct, rapid (15 min) effect of 1,25(OH)₂D₃ on sarcomere shortening (figure 3). Peak shortening was markedly decreased as early as 2.5 min after addition of 1 nM of 1, 25 (OH)₂D₃ (decreased 11%, P<0.05 from control), while at 30 min a decrease of 28% (P<0.01 from control) of sarcomere shortening was observed (figure 4). These data clearly demonstrated a rapid effect of 1,25(OH)₂D₃ on cardiomyocyte contractility. Overall our data support a rapid activation by 1,25(OH)₂D₃ of the VDR that was found to be associated with the sarcolemma and t-tubules of cardiac myocytes.

Discussion

The rapid, non-genomic actions of vitamin D3 have been reported and it is thought that 1, 25 (OH)₂D₃ exerts its effects through a membrane associated VDR, although its characterization remains incomplete (7-10). It has been shown that VDR is associated with caveolae-1 micro domains in chick intestinal cells, suggesting a possible mechanism of membrane localization (11). In accordance, our lab has reported that the t-tubule fraction of rat hearts to be enriched in VDR and caveolin-3, the predominant caveolin subtype in cardiac myocytes (12). In this study, we demonstrated that VDR is physically associated with Caveolin-3 in the t-tubules and sarcolemma of the cardiomyocyte by confocal immunofluorescence microscopy studies and

co-immunoprecipitation studies. We have also shown a rapid direct effect of $1, 25(\text{OH})_2\text{D}_3$ on contraction in isolated individual cardiomyocyte, which is to decrease the stimulated peak of contraction or sarcomere shortening. We found that this effect was significant within 2.5 min of and maximum at 10-15 min of treatment with $1, 25(\text{OH})_2\text{D}_3$. This effect may be attributed to the membrane-associated VDR given the facts that the effect was seen within minutes of $1, 25(\text{OH})_2\text{D}_3$ treatment and the localization of this VDR to the t-tubule system that is critical for cardiomyocyte control of calcium trafficking.

The finding of membrane-associated VDR interacting with Caveolin-3 in cardiac myocytes is relevant and important. Caveolin is the principle structural protein that is both necessary and sufficient for the formation of the caveolae membrane domain. Caveolae function both in protein trafficking and signal transduction, as well as in cholesterol homeostasis [14,15]. In addition to a structural role of caveolins in the formation of caveolae, caveolin proteins interact directly with a number of signaling molecules involved in various signaling pathways and modify their functions. These include the Src-family tyrosine kinase, MAP kinase cascades, adenylyl cyclase, PKA, PKC, TGF receptor pathway, and endothelial nitric oxide synthase (eNOS) [18]. Caveolin holds these signal transducers in the inactive conformation until activation by an appropriate stimulus. Thus, it is possible that VDR could exert its effects through its interaction with Caveolin-3 and by affecting caveolin-associated signal transduction pathways. Interestingly, $1, 25(\text{OH})_2\text{D}_3$ increased inward barium currents through L-type Ca channels of osteoblasts at low depolarizing potentials within seconds in a fashion similar to the 1,4-dihydropyridine [DHP] agonist Bay K8644 (23). The possible physiological significance of the rapid effect we describe here is already apparent. To date over 24 clinical studies have been conducted. As recently reviewed, these studies examined the relationship between vitamin D levels and cardiovascular disease and they have shown that lower vitamin D levels are associated with an increased risk of cardiovascular disease (1).

It is the rate of calcium influx through calcium channels that is responsible for the rate of cardiomyocyte contraction. These calcium channels are located primarily at the t-tubules. T-tubules are composed of interconnected caveolae-like elements, and repeated caveolae formation in the absence of fission leads to the generation of t-tubules [19]. Many of the proteins involved in excitation-contraction coupling are concentrated at the t-tubules, including L-type calcium channels (LTCC), which play a critical role in regulating calcium-dependent signaling in cardiac myocytes [20]. The β_2 -adrenergic receptor, associated with caveolin-3 in cardiac myocytes, is important in the regulation of a number of key proteins in the heart, in particular, the LTCC, the SR Ca^{2+} -ATPase, and the contractile proteins (via troponin I) [21]. Recently it has been shown that subcellular localization of LTCC to caveolar macromolecular signaling complexes is essential for regulation of the channels by the β_2 -adrenergic receptor [22]. Previous studies in our lab have shown an increased nuclear localization of VDR after $1, 25(\text{OH})_2\text{D}_3$ treatment in cardiomyocytes (12). It is now reasonable to hypothesize that Vitamin D_3 and $1, 25(\text{OH})_2\text{D}_3$ treatment could cause changes (increase or decrease the association) in the interaction between VDR and caveolin-3, thus causing a cascade effect on caveolin-associated signal transducers, that then affect the contraction of cardiac myocytes.

References

1. Nemerovski CW, Dorsch MP, Simpson RU, Bone HG, Aaronson KD, Bleske BE. Vitamin D and cardiovascular disease. *Pharmacotherapy* Jun;2009 29(6):691–708. [PubMed: 19476421]
2. Weishaar RE, Simpson RU. Vitamin D_3 and cardiovascular function in rats. *J Clin Invest* 1987;79:1706–12. [PubMed: 3034981]
3. Shane E, Mancini D, Aaronson K, Silverberg SJ, Seibel MJ, Adesso V, et al. Bone mass, vitamin D deficiency, and hyperparathyroidism in congestive heart failure. *Am J Med* 1997;103:197–207. [PubMed: 9316552]

4. O'Connell TD, Simpson RU. 1,25-Dihydroxyvitamin D3 regulation of myocardial growth and c-myc levels in the rat heart. *Biochem Biophys Res Commun* 1995;213:59–65. [PubMed: 7639762]
5. Weishaar RE, Kim SN, Saunders DE, Simpson RU. Involvement of vitamin D3 with cardiovascular function. III. Effects on physical and morphological properties. *Am J Physiol* 1990;258:E134–42. [PubMed: 2154115]
6. Simpson RU, Hershey SH, Nibbelink KA. Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. *J Steroid Biochem Mol Biol* 2007;103:521–4. [PubMed: 17275289]
7. De Boland AR, Boland RL. Non-genomic signal transduction pathway of vitamin D in muscle. *Cell Signal* 1994;6:717–24. [PubMed: 7888298]
8. Morelli S, Buitrago C, Boland R, de Boland AR. The stimulation of MAP kinase by 1,25(OH)(2)-vitamin D(3) in skeletal muscle cells is mediated by protein kinase C and calcium. *Mol Cell Endocrinol* 2001;173:41–52. [PubMed: 11223176]
9. Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology* 2006;147:5542–8. [PubMed: 16946007]
10. Capiati D, Benassati S, Boland RL. 1,25(OH)2-vitamin D3 induces translocation of the vitamin D receptor (VDR) to the plasma membrane in skeletal muscle cells. *J Cell Biochem* 2002;86:128–35. [PubMed: 12112023]
11. Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 alpha, 25(OH)2-vitamin D3 in vivo and in vitro. *Mol Endocrinol* 2004;18:2660–71. [PubMed: 15272054]
12. Tishkoff DX, Nibbelink KA, Holmberg KH, Dandu L, Simpson RU. Functional Vitamin D Receptor in the T-Tubules of Cardiac Myocytes. *Endocrinology*. 2007
13. Razani B, Woodman SE, Lisanti MP. Caveolae: from cell biology to animal physiology. *Pharmacol Rev* 2002;54:431–67. [PubMed: 12223531]
14. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000;1:31–9. [PubMed: 11413487]
15. Galbiati F, Razani B, Lisanti MP. Emerging themes in lipid rafts and caveolae. *Cell* 2001;106:403–11. [PubMed: 11525727]
16. Green JJ, Robinson DA, Wilson GE, Simpson RU, Westfall MV. Calcitriol modulation of cardiac contractile performance via protein kinase C. *J Mol Cell Cardiol* 2006;41:350–9. [PubMed: 16815434]
17. Nibbelink KA, Tishkoff DX, Hershey SD, Rahman A, Simpson RU. 1,25(OH)2-vitamin D3 actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. *J Steroid Biochem Mol Biol* 2007;103:533–7. [PubMed: 17276054]
18. Williams TM, Lisanti MP. The Caveolin genes: from cell biology to medicine. *Ann Med* 2004;36:584–95. [PubMed: 15768830]
19. Franzini-Armstrong C. Simultaneous maturation of transverse tubules and sarcoplasmic reticulum during muscle differentiation in the mouse. *Dev Biol* 1991;146:353–63. [PubMed: 1864461]
20. Takagishi Y, Yasui K, Severs NJ, Murata Y. Species-specific difference in distribution of voltage-gated L-type Ca(2+) channels of cardiac myocytes. *Am J Physiol Cell Physiol* 2000;279:C1963–9. [PubMed: 11078712]
21. Ostrom RS, Violin JD, Coleman S, Insel PA. Selective enhancement of beta-adrenergic receptor signaling by overexpression of adenylyl cyclase type 6: colocalization of receptor and adenylyl cyclase in caveolae of cardiac myocytes. *Mol Pharmacol* 2000;57:1075–9. [PubMed: 10779394]
22. Balijepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ. Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation. *Proc Natl Acad Sci USA* 2006;103:7500–5. [PubMed: 16648270]
23. Zanello LP, Norman A. 1alpha, 25(OH)2 vitamin D3 actions on ion channels in osteoblasts. *Steroids* 2006;(4):291–7. [PubMed: 16457860]

Figure 1.

Immunofluorescent confocal microscopy of adult rat cardiac myocytes. Double labeling using an antibody against VDR (sc-1008, rabbit polyclonal, a) and an antibody against caveolin-3 (sc-5310, mouse monoclonal, b) show similar transverse staining pattern, whereas a merge (c) shows association.

Figure 2 A.

Co-immunoprecipitation of caveolin-3 with VDR antibodies. 600 ug of cardiomyocyte and intestinal protein lysates were incubated with VDR monoclonal and polyclonal antibody-coupled gel resins and control gel resin. Eluted fractions of protein samples (20 ul each) were subjected to Western blot analysis. The blot was first probed with Caveolin-3 antibody (sc-5310) and then striped, and re-probed with VDR antibody (sc-1008).

CO-IP of VDR with Cav3 in Rat Cardiomyocytes

Figure 2B.

Co-immunoprecipitation of VDR with Caveolin-3 antibody. 600 ug of cardiomyocyte lysates were incubated with caveolin-3 antibody-coupled gel resin and control resin. Eluted fractions of immunoprecipitated samples (20 ul each) were subjected to Western blot analysis. The blot was first probed with Caveolin-3 antibody (sc-5310) and then striped, and re-probed with VDR antibody (sc-1008).

Figure 3.

Average normalized sarcomere shortening trace of adult rat cardiomyocytes under basal conditions (n=4), and after 15-min treatment with 1 nM 1,25(OH)₂D₃ (n=4). All the sarcomere lengths at different time points were normalized to the sarcomere length at time 0 for each cell. Each tracing is the cumulative average of 40 contraction events in 4 hearts cardiomyocytes.

Figure 4.

Time course for percent change in peak shortening in response to 1 nM 1,25(OH)₂D₃ treatment. Isolated individual rat cardiomyocyte was electrically stimulated and recorded before and after 1,25(OH)₂D₃ treatment. Data shown are presented as percent change in peak height after treatment compared to peak height before treatment (time zero). Data shown were average of 4 cells and 40 contraction events from rat hearts.