1,25-Dihydroxyvitamin D and 25-hydroxyvitamin D – mediated regulation of TRPV6 (a putative epithelial calcium channel) mRNA expression in Caco-2 cells

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**Summary** Background TRPV6 is a member of the vanilloid subfamily of transient receptor potential (TRP) proteins and likely functions as an epithelial calcium channel in calcium-transporting organs, such as the intestine, kidney, and placenta. TRPV6 mRNA expression is strongly regulated by 1,25-dihydroxyvitamin D (1,25VD), the active hormonal form of vitamin D, in intestine and in Caco-2 cells, a human colon cancer cell line. Aim of the study The aim of the present study was to characterise the mode of regulation of the 1,25VD-mediated TRPV6 mRNA expression and to test the effect of the precursor of 1,25VD namely 25 hydroxyvitamin D (25VD) on TRPV6 mRNA expression in Caco-2 cells. Methods Caco-2 cells were treated in a 2 x 2 format with 1,25VD and the transcriptional inhibitor actinomycin D (AD, 4 µg/ml), and also with translational inhibitor cycloheximide (CHX, 10 µg/ml) after 14 days in culture and TRPV6 mRNA levels were determined using reverse transcription-real time PCR. TRPV6 mRNA half life studies were performed by inhibiting transcription followed by sampling at various time points for TRPV6 mRNA. Varying concentrations of 25VD were used to test their effect on TRPV6 mRNA in the presence of 5 % FBS and also in the absence of serum (but containing insulin-transferrin-selenium mixture) for 24 h. Results Treatment with 10⁻⁷ M 1,25VD for 8 h resulted in a 60-fold increase in TRPV6 mRNA and this increase could be completely blocked with AD. Treatment with CHX to inhibit de novo protein synthesis did not prevent the initiation of 1,25VD-induced TRPV6 expression, although it did reduce the extent of TRPV6 mRNA accumulation. We found that TRPV6 mRNA half-life was 8 h in Caco-2 cells and was not altered by 1,25VD treatment. Finally, we observed that treatment with 10⁻⁶ M of the pro-hormone 25VD for 24 h resulted in a significant increase in TRPV6 expression in Caco-2 cells, which is consistent with the presence of 1α-hydroxylase (CYP27B1) expression in Caco-2 cells and a possible autocrine vitamin D signaling pathway in colon cells. Conclusions 1,25 dihydroxyvitamin D regulates TRPV6 expression by a process that requires new mRNA and protein synthesis and the point of regulation lies likely at the transcriptional level especially since vitamin D did not increase the half life of TRPV6 mRNA. In addition, the prohormone form of 1,25 dihydroxyvitamin D, i.e. the 25 hydroxyvitamin D, induced TRPV6 mRNA expression in Caco-2 cells.

**Key words** CaT1 – TRPV6 – gene expression – vitamin D – intestine
Introduction

1,25-dihydroxyvitamin D is the primary hormonal regulator of intestinal calcium absorption by a process involving an increase in transcellular calcium transport [1]. However, the molecular mechanism of calcium transport across the enterocyte has not been fully described. Three important steps in transcellular calcium transport include: calcium influx across the apical brush border membrane, by an as yet unidentified transporter; transcytosis of absorbed calcium, likely by calbindin D₉k, a vitamin D-dependent cytosolic mobile calcium buffer; and finally, calcium extrusion across the basolateral membrane by an energy-dependent process involving calcium ATPase [1,2].

The transient receptor potential (TRP) family of proteins function as plasma membrane channels that mediate important cellular functions such as osmoregulation, sensory response to touch, hot and cold temperature [3]. TRPV6 (transient receptor potential channel, subfamily V, member 6) gene encodes an epithelial calcium channel namely CaT1 (calcium transporter-1) which was first identified by Peng and colleagues [4]. It is an integral membrane protein with six membrane-spanning domains and a pore region for ion transport [5]. TRPV6 mRNA was first shown to be regulated by 1,25-dihydroxyvitamin D [6] in Caco-2 cells, a human intestinal cell line. 1,25-dihydroxyvitamin D is also known to increase transcellular calcium transport in Caco-2 cells [7,8]. Thus, we have proposed that gene product of TRPV6 is a candidate molecular gatekeeper for vitamin D-dependent calcium entry into the enterocyte [6]. The dependence of TRPV6 mRNA expression on the VDR-mediated genomic actions of 1,25-dihydroxyvitamin D is supported by increased intestinal TRPV6 mRNA expression after injection of mice with 1,25-dihydroxyvitamin D [9,10] and that TRPV6 expression was lower in vitamin D receptor knockout mice as compared to wild type mice [10]. However, the mode of 1,25-dihydroxyvitamin D-mediated regulation of TRPV6 expression is unknown. It is not known whether 1,25-dihydroxyvitamin D increases the abundance of TRPV6 transcripts merely by de novo transcription or also by decreasing the degradation rate of TRPV6 mRNA and whether or not new protein synthesis is required for 1,25-dihydroxyvitamin D to upregulate TRPV6 mRNA. Transcriptional activation and repression of vitamin D-dependent genes is a primary mechanism of action of 1,25-dihydroxyvitamin D in the cell [11]. However, several studies have shown that 1,25-dihydroxyvitamin D is also involved in post-transcriptional regulation [12–18] by altering mRNA half-life of vitamin D-dependent genes. For example, 1,25-dihydroxyvitamin D increases the expression of epidermal growth factor receptor (EGFR) exclusively by increasing mRNA half life [12], while 1,25-dihydroxyvitamin D increases both the transcription rate and mRNA half life of osteocalcin [14] and collagenase [15]. Therefore, we hypothesised that 1,25-dihydroxyvitamin D may also stabilise the TRPV6 transcript.

Plasma 1,25-dihydroxyvitamin D concentration is determined by the availability of the pro-hormone 25-hydroxyvitamin D and the activity of the renal 1α-hydroxylase. The conversion of 25-hydroxyvitamin D (the pro-hormone form of vitamin D) to the active 1,25-dihydroxyvitamin D hormone is tightly controlled by the renal 1α-hydroxylase enzyme. However, some non-renal cells, including those of the prostate [19], colon [20], pancreas [21], placenta [22], parathyroid gland [23] and cervix [24] also express 1α-hydroxylase, which may result in the local conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, setting up the possibility of an autocrine or paracrine vitamin D signaling pathway in these tissues [20].

The objective of this study was to characterise the mode of regulation of 1,25-dihydroxyvitamin D-mediated TRPV6 mRNA expression and also to test the effect of 25-hydroxyvitamin D (a precursor of 1,25 vitamin D) on TRPV6 mRNA expression in human intestinal Caco-2 cells.

Materials and methods

Materials

Caco-2 cells were obtained from American Type Culture Collection (HTB37; American Type Culture Collection, Rockville, MD). 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D were purchased from Biomol, Plymouth Meeting, PA. Actinomycin D and cycloheximide were from Sigma, St. Louis, MO. TRF reagent and bromochloropropane for RNA isolation were purchased from Molecular Research Center, Cincinnati, OH. Moloney-murine leukemia virus reverse transcriptase enzyme was bought from Invitrogen, Carlsbad, CA. SYBR Green master mix (2X) for real time PCR was from Applied Biosystems, Foster City, CA. Fetal bovine serum (FBS) was from Hyclone, Logan UT. Plastic six-well (35-mm diameter) cell culture dishes were from Costar, Cambridge, MA. Other cell culture materials were purchased from Invitrogen, Carlsbad, CA.

Cell culture

Caco-2 cells were cultured at 37°C in a humid 5% CO₂ and 95% air atmosphere. Cells used in these studies were between passages 30 to 50. The maintenance medium for the cells consisted of high glucose (4.5 g/L glucose) Dulbecco’s Modified Eagle Medium (DMEM),