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Regulation of the epithelial Ca^{2+} channels TRPV5 and TRPV6 by 1α,25-dihydroxy Vitamin D_3 and dietary Ca^{2+}☆

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Abstract

Active, transepithelial Ca^{2+} transport is a pivotal process in the regulation of Ca^{2+} homeostasis and consists of three sequential steps: apical Ca^{2+} influx, diffusion towards the basolateral membrane and subsequent extrusion into the blood compartment. TRPV5 and TRPV6 (renamed after ECaC1 and ECaC2/CaT1, respectively) constitute the rate-limiting influx step of transepithelial Ca^{2+} transport and these highly selective Ca^{2+} channels are controlled by several factors. This review focuses on the regulation of TRPV5 and TRPV6 abundance and/or activity by 1α,25-dihydroxyVitamin D_3 (1α,25$(OH)_2D_3$), dietary Ca^{2+} and the auxiliary protein pair S100A10/annexin 2. Finally, the implications for our understanding of transcellular Ca^{2+} transport will be discussed.

Keywords: Calcitriol; Calcium reabsorption; ECaC; CaT1

1. Introduction

The maintenance of the body Ca^{2+} balance is of crucial importance for many vital physiological functions including neuronal excitability, muscle contraction and bone formation. Total body Ca^{2+} homeostasis is tightly controlled by a concerted action of intestine, kidney and bone. In the kidney, ∼8 g Ca^{2+} is filtered at the glomerulus on a daily basis, of which <2% is excreted into the urine. The majority of Ca^{2+} reabsorption occurs via passive paracellular transport in the proximal tubules and the thick ascending loop of Henle [1]. Only ∼15% is reabsorbed via an active transcellular Ca^{2+} transport route in the distal part of the nephron [1,2]. Similarly, intestinal Ca^{2+} absorption consists of both a passive paracellular and active transcellular pathway [3], whereas the molecular nature of Ca^{2+} transport in bone remains largely elusive. The only target site for specific regulation of Ca^{2+} (re)absorption is the transcellular pathway in kidney and intestine [4,5]. Transcellular Ca^{2+} transport can be divided in three sequential steps [3,6]. First, Ca^{2+} enters the cell from the apical lumen. The second step is intracellular diffusion to the basolateral side of the cell. During this process Ca^{2+} is tightly bound to the Ca^{2+}-binding proteins calbindin-D_{9K} and/or calbindin-D_{28K}. Finally, Ca^{2+} is extruded at the basolateral side of the cell by two Ca^{2+} extrusion pathways: the plasma membrane Ca^{2+} ATPase PMCA1b and the Na^+/Ca^{2+} exchanger (NCX1) [6]. NCX1 function is limited to the kidney, whereas PMCA1b is present in kidney and intestine, although it has a more prominent function in the latter organ [7]. Apical Ca^{2+} influx is the rate-limiting step of the whole process and is, therefore, the most efficient target for regulation. The molecular identity of the apical Ca^{2+} entry pathway remained elusive until the identification of the epithelial Ca^{2+} channel, initially named ECaC1 [8]. Thereafter, a second distinct, but highly homologous, epithelial Ca^{2+} channel was identified from rat duodenum, and was named CaT1 or ECaC2 [9]. These two channels comprise a new subfamily of the transient receptor potential (TRP) family and display the highest sequence homology with the vanilloid receptor subfamily (TRPV) of these channels. Therefore, ECaC1 and ECaC2 were recently renamed into TRPV5 and TRPV6, respectively [10]. Southern blot analysis, using the conserved pore area as probe, demonstrated that the epithelial Ca^{2+} channel family is restricted to these two members [11]. Expression profiling of TRPV5 and TRPV6 using Northern blotting, RT-PCR analysis and immunohistochemistry showed expression in kidney, small intestine, placenta, prostate, pancreas, salivary gland, brain, colon and rectum [8,9,12–14]. Although TRPV5 and TRPV6 are co-expressed in several of these tis-

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sues, their relative expression needs to be further resolved. In general, TRPV5 seems to be the more significant isoform in kidney, whereas TRPV6 is prominently expressed in small intestine and prostate. TRPV5 has been localized immunohistochemically to the apical domain of the late part of the distal convoluted tubule (DCT2) and connecting tubules (CNT) in the kidney cortex [15]. In all tissues, TRPV5 completely co-localized with the proteins known to be involved in active Ca^{2+} transport. Immunohistological studies demonstrated the localization of TRPV6 in the brush border membrane of intestinal absorptive cells [16]. These findings hint at a role of these channels as the rate-limiting apical influx pathway of active Ca^{2+} transport. Interestingly, it was recently shown that TRPV5 and TRPV6 operate as homo- and hetero-tetrameric Ca^{2+} channels and, depending on their composition, display distinct electrophysiological characteristics [17]. The co-expression of both channels in kidney and duodenum suggests that these heterotetramers are also formed in vivo. Controlling the relative contribution of both channels in the tetrameric complex, would enable the organism to adapt characteristics of the epithelial Ca^{2+} channel complex and, thereby, to fine-tune transepithelial Ca^{2+} transport. Understanding the regulation of TRPV5 and TRPV6 can, therefore, be instrumental for our insight in the molecular mechanisms of hormonally regulated transcellular Ca^{2+} transport. This review highlights the pivotal role of Vitamin D, dietary Ca^{2+} and channel-interacting proteins in the regulation of these two epithelial Ca^{2+} channels.

2. Regulation of TRPV5 and TRPV6 by auxiliary proteins

Recently, S100A10 and annexin 2 were identified as novel TRPV5 and TRPV6 auxiliary proteins [18]. Annexin 2 forms a well-defined heterotetrameric complex with S100A10, a distinct Ca^{2+}-insensitive member of the S100 family. Association of annexin 2 with the TRPV5 C-terminal tail could only be demonstrated in the presence of S100A10, suggesting that S100A10 operates as a bridge between TRPV5 and annexin 2. The binding of S100A10 could be localized to a short, highly conserved, stretch in the C-terminal tail of TRPV5. Disruption of this S100A10-binding motif in TRPV5 and TRPV6 resulted in abolishment of channel activity. This effect was accompanied by a major disturbance in the subcellular localization of TRPV5 and TRPV6. Furthermore, downregulation of annexin 2 using annexin 2-specific short interfering RNA (siRNA) significantly inhibited TRPV5- and TRPV6-mediated currents. Together this demonstrated that the S100A10–annexin 2 complex is an important component for the trafficking of TRPV5 and TRPV6 to the plasma membrane [18]. Besides regulation by channel-associated proteins, TRPV5 and TRPV6 activity is under control of calciotrophic hormones, as discussed below.

3. (Patho)physiological role of Vitamin D

The key regulator of Ca^{2+} (re)absorption is $1\alpha,25(OH)_2D_3$, the biologically active form of Vitamin D. $1\alpha,25(OH)_2D_3$ enhances the active Ca^{2+} absorption in small intestine and stimulates Ca^{2+} reabsorption in kidney [1,3,4]. The importance of this hormone is reflected by several disorders that are linked to genes involved in Vitamin D activity. Vitamin D-deficiency rickets type I (VDDR-I) is an autosomal recessive hereditary defect in Vitamin D metabolism, characterized by low or undetectable levels of $1,25(OH)_2D_3$ [19]. Hypophosphatemia, growth retardation, and rickets or osteomalacia are the major clinical findings [19]. The disease is caused by a mutation on chromosome 12q14 that affects the gene encoding 25-hydroxyVitamin D_3-1α-hydroxylase (1α-OHase), which converts the inactive metabolite 25-hydroxyVitamin D_3 into $1\alpha,25(OH)_2D_3$ in the kidney [20]. The second Vitamin D-related disease is hereditary hypocalcemic Vitamin D-dependent rickets type II (VDDR-II). Patients with VDDR-II display a similar phenotype as VDDR-I patients but the first have elevated serum $1\alpha,25(OH)_2D_3$ levels [21]. VDDR-II presents with congenital body alopecia and onset of rickets during the second half of the first year of life and patients display rapidly progressive rachitic bone changes, hypocalcemia and secondary hyperparathyroidism. VDDR-II is caused by mutations in the gene encoding the nuclear Vitamin D receptor (VDR), which mediates the biological activity of $1\alpha,25(OH)_2D_3$ [21]. Experiments performed with VDR knockout mice revealed disturbed Ca^{2+} absorption and inappropriately high renal Ca^{2+} excretion, consistent with a target tissue insensitivity to Vitamin D [22,23]. Examples of non-hereditary forms of Vitamin D-related Ca^{2+} homeostasis disorders are osteoporosis in elderly people and non-hereditary rickets in infants. The main cause of rickets is inadequate Vitamin D supply resulting from prolonged breast feeding without Vitamin D supplementation and concomitant avoidance of sun exposure [24]. Furthermore, the relative risk of osteoporosis in elderly is largely decreased by Vitamin D and Ca^{2+} administration, further demonstrating the significance of Vitamin D in the (patho)physiology of Ca^{2+} homeostasis [25]. Many studies described effects of $1\alpha,25(OH)_2D_3$ that are not mediated by the nuclear Vitamin D receptor, pointing to the existence of a non-genomic pathway of $1\alpha,25(OH)_2D_3$ stimulation. Non-genomic effects of $1\alpha,25(OH)_2D_3$ occur in the seconds to minutes timescale and are mediated by a putative membrane bound receptor. Remarkably, Baran et al. identified annexin 2 as the Vitamin D analog-binding protein present in plasma membranes of rat osteoblast-like cells (ROS 24/1) [26], suggesting that annexin 2 is the putative membrane bound receptor involved in rapid biological responses to $1\alpha,25(OH)_2D_3$ stimulation. However, antibodies generated against a $[^3H]1,25(OH)_2D_3$ binding protein of 66 kDa failed to recognize annexin 2 (36 kDa), although they were able to block the $1\alpha,25(OH)_2D_3$-dependent effects in chondrocytes, suggesting the presence of a putative

Vitamin D-binding receptor other than annexin 2 [27,28]. Recently, the Vitamin D-dependent regulation of TRPV5 and TRPV6 was described, which provided a more detailed understanding of the molecular mechanisms of Vitamin D efficacy.

4. Regulation of TRPV5 and TRPV6 by Vitamin D

Initial evidence for the Vitamin D-sensitivity of TRPV5 was obtained in studies where rats were raised on a Vitamin D-depleting diet [29]. Concurrently with the serum 1,25-dihydroxyVitamin D_3 levels, mRNA and protein levels of TRPV5 decreased significantly. Repletion of serum $1\alpha,25(OH)_2D_3$, via intraperitoneal injections, resulted in a significant upregulation of TRPV5 to control levels. This was accompanied by a normalization of serum Ca^{2+} levels [29]. In a subsequent paper from Wood et al. the ability of $1\alpha,25(OH)_2D_3$ to regulate the expression of TRPV6 in vitro was described [30]. Caco-2 cells, which are used as a human intestinal cell culture model, were shown to endogenously express TRPV6. Administration of $1\alpha,25(OH)_2D_3$ to these cells resulted in an increase of TRPV6 expression within 24 h, which preceded an increase in expression levels of calbindin-D_{9K}. Similar doses of $1\alpha,25(OH)_2D_3$ have been shown to stimulate Ca^{2+} transport across confluent layers of these Caco-2 cells, in line with a role for TRPV6 as the gatekeeper of transepithelial Ca^{2+} transport [30]. Administration of $1\alpha,25(OH)_2D_3$ to mice increased the expression levels of TRPV5, TRPV6 and calbindin-D_{9K} in duodenum and kidney, further confirming the $1\alpha,25(OH)_2D_3$-dependent regulation of these Ca^{2+} transport proteins [23]. Analysis of the putative promoter regions in the human and murine TRPV5 and TRPV6 genes has revealed potential Vitamin D responsive elements, providing an explanation for the Vitamin D-mediated increase in channel expression [14,29,31]. To date, rapid responses of $1\alpha,25(OH)_2D_3$ on TRPV5 or TRPV6 activity have not been reported, suggesting that the mechanism of $1\alpha,25(OH)_2D_3$ efficacy on transepithelial Ca^{2+} transport represent the nuclear VDR-mediated pathway.

5. Impaired TRPV5 and TRPV6 expression in Vitamin D receptor knockout mice

Vitamin D receptor (VDR) knockout mice display a phenotype similar to Vitamin D-resistant rickets (VDDR type II) and are, therefore, useful mouse models to study the molecular mechanisms associated with this disease [22]. Van Cromphaut and coworkers demonstrated that duodenal expression of TRPV5 and TRPV6 was drastically down-regulated in two independent VDR knockout mouse models [23]. The expression levels of other Ca^{2+} transporting proteins were not or only moderately decreased in the VDR knockout mice compared to wild-type mice. Furthermore, Weber et al. showed that renal TRPV5 and duodenal TRPV6 expression levels were reduced in a VDR knockout mouse model [14]. Together, these data suggest that TRPV5 and TRPV6 can be crucial factors in the VDR knockout mouse or the human VDDR-II phenotype.

6. TRPV5 and TRPV6 regulation in 1-α-OHase deficient mice

Recently, a 1α-OHase knockout mouse strain was generated by Dardenne et al. by targeted inactivation of the 1α-OHase gene [32]. This mouse is a valuable animal model for VDDR-I, as they display undetectable levels of $1\alpha,25(OH)_2D_3$, hypocalcemia and secondary hyperparathyroidism. Furthermore, they show pronounced histological evidence of rickets. The 1α-OHase knockout mouse was used to investigate the regulation of several Ca^{2+} transport proteins including TRPV5 and TRPV6 and their role in Ca^{2+} homeostasis. Down-regulation of renal TRPV5 as well as intestinal TRPV6 expression was demonstrated in the knockout mice, compared to wild-type littermates [33]. These effects were accompanied by decreased calbindin and NCX1 levels. Supplementation of $1,25(OH)_2D_3$ restored expression levels of these Ca^{2+} transporters, resulting in normalization of serum Ca^{2+} levels. Remarkably, annexin 2 expression levels have been shown to increase upon prolonged incubation with $1\alpha,25(OH)_2D_3$ [34]. Furthermore, S100A10 abundance is, analogous to TRPV5 and TRPV6, regulated by $1\alpha,25(OH)_2D_3$ in kidney [18,33]. These observations imply that $1\alpha,25(OH)_2D_3$ exerts a major calciotrophic effect by concomitantly increasing the expression of S100A10, annexin 2 and the epithelial Ca^{2+} channels TRPV5 and TRPV6 (Fig. 1) and thus efficiently stimulates Ca^{2+}-channel recruitment during Ca^{2+} demand.

7. Influence of dietary Ca^{2+} on TRPV5 and TRPV6 expression

Rickets and hyperparathyroidism caused by a defective Vitamin D receptor can be prevented in humans and animals by high Ca^{2+} intake, suggesting a VDR-independent pathway on Ca^{2+} homeostasis [23]. Rescue of the hypocalcemic state of the VDR knockout mice was accompanied by a slight increase in renal TRPV6 expression [23]. However, in duodenum, different effects were reported. In wild-type animals, high dietary Ca^{2+} content decreased the expression of both TRPV5 and TRPV6 [23]. This effect was accompanied by a large decrease in $1\alpha,25(OH)_2D_3$ concentration in the blood. Rapid effects of $1\alpha,25(OH)_2D_3$ have been described in several cell tissues including intestine and kidney [35,36] and are accompanied by increases in cytosolic Ca^{2+}, pH and cyclic nucleotides and PKC activation [26]. It is conceivable that some of these pathways will influence the expression of TRPV5 or TRPV6 [33]. Therefore,

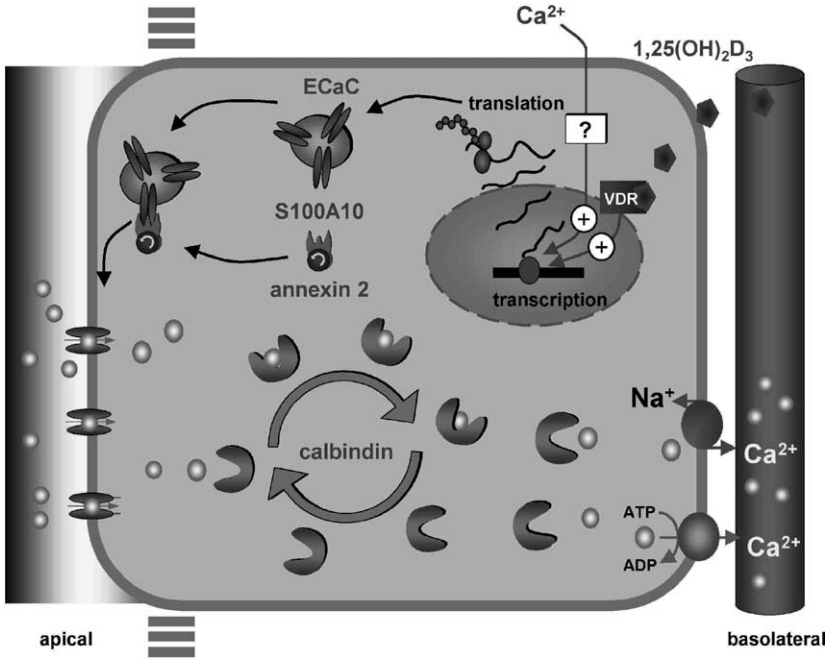

Fig. 1. Integrated model of active Ca^{2+} (re)absorption. Apical Ca^{2+} influx is mediated by TRPV5 or TRPV6, Ca^{2+} then binds to calbindin and is transported to the basolateral membrane, where it is extruded by a plasma membrane Ca^{2+}-ATPase and a Na^+/Ca^{2+} exchanger. Regulation TRPV5 and TRPV6 activity involves $1\alpha,25(OH)_2D_3$-mediated channel transcription/translation, channel shuttling and insertion into the plasma membrane mediated by channel-associated proteins, including S100A10 and annexin 2.

1α-OHase knockout mice are an ideal model to study the role of dietary Ca^{2+} in the regulation of TRPV5 or TRPV6 independent of $1\alpha,25(OH)_2D_3$. High dietary Ca^{2+} treatment results in the rescue of the 1α-OHase knockout mice from several aspects of the VDDR-I like phenotype, including hypocalcemia. Interestingly, high dietary Ca^{2+} intake, which down-regulated renal TRPV5 expression in wild-type mice, resulted in an upregulation of renal TRPV5 and the proteins involved in active Ca^{2+} transport in 1α-OHase knockout mice [33]. Furthermore, in duodenum, TRPV6, calbindin-D_{9K} and PMCA1b are increased upon high dietary Ca^{2+} treatment in the 1α-OHase knockout mice, demonstrating the $1\alpha,25(OH)_2D_3$-independent regulation of Ca^{2+} transport proteins by dietary Ca^{2+} [37]. These data further suggest that decreased channel abundance contributes to defective renal and intestinal Ca^{2+} absorption and, thereby, the sustained hypocalcemia in VDDR-I. Rescue by high dietary Ca^{2+} of this phenotype corresponds with an increase of the apical Ca^{2+} influx channels, suggesting an important role for TRPV5 and TRPV6 in the rescue mechanism of these animals.

8. Conclusions

Active transcellular Ca^{2+} transport involves a chain of Ca^{2+} transport proteins involved in apical Ca^{2+} influx, transport to the basolateral membrane and extrusion into he bloodstream. Regulation of the two channels that mediate the rate-limiting Ca^{2+} entry step, TRPV5 and TRPV6, is pivotal to control the Ca^{2+} transport rate. In this respect, elucidation of the mechanisms underlying the activity of TRPV5 and TRPV6 is significant for our understanding of the molecular nature of Ca^{2+} homeostasis. Several lines of

Table 1
Regulation of TRPV5 and TRPV6 by Vitamin D and dietary Ca^{2+} in kidney and small intestine

Vitamin D	WT + Vit. D	1α-OHase−/−	1α-OHase−/− + Vit. D	VDR knockout	Dietary Ca^{2+}	WT	1α-OHase−/−
Kidney					Kidney		
TRPV5	↑	↓	↑	=↓	TRPV5	↓	↑
TRPV6	↑	?	?	=	TRPV6	?	?
Duodenum					Duodenum		
TRPV5	↑	↓	↑	↓	TRPV5	↓	ND
TRPV6	↑	↓	↑	↓	TRPV6	↓	↑
References	[23,29]	[33,37]	[33,37]	[14,23]	[23,33]	[33,37]	

ND, not detectable; (?) not determined; (=) not changed.

evidence suggest that the biological activity TRPV5 and TRPV6 is regulated by various factors [5]. Firstly, TRPV5 and TRPV6 abundance is regulated by dietary Ca^{2+} and $1\alpha,25(OH)_2D_3$ as summarized in Table 1. Secondly, TRPV5 and TRPV6 are regulated by the channel-associated proteins S100A10 and annexin 2. The abundance of these auxiliary proteins are also regulated by $1\alpha,25(OH)_2D_3$. Despite progress in the research of these channels, many aspects of TRPV5 and TRPV6 regulation remain elusive. Further research will be essential to unravel the role of TRPV5 or TRPV6 in Ca^{2+} homeostasis-related disorders, to identify channel regulatory mechanisms and to find pharmaceutics that influence the channel activity.

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