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Regulation of gene expression of epithelial calcium channels in intestine and kidney of mice by 1α,25-dihydroxyvitamin D_3 ☆

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Abstract

In wild-type ($VDR^{+/+}$) mice, ECaC2 expression was confirmed in the intestine and kidney, while ECaC1 expression was exclusively confined to the kidney. Both mRNAs expression of ECaC1 and ECaC2 in the kidney and ECaC2 mRNA expression in the intestine increased time- and dose-dependently in response to 1α,25$(OH)_2D_3$ injection in $VDR^{+/+}$ mice, but not in $VDR^{-/-}$ mice. The mRNA levels of ECaC2 in the intestine of $VDR^{-/-}$ mice were remarkably reduced when compared to $VDR^{+/+}$ mice, while no significant differences were observed in both mRNA levels of ECaC1 and ECaC2 in the kidney between $VDR^{+/+}$ mice and $VDR^{-/-}$ mice. In the primary renal tubular cells (PRTC) isolated from $VDR^{+/+}$ mice, both ECaCs mRNA expression increased in response to 1α,25$(OH)_2D_3$ treatment, but not in the PRTC of $VDR^{-/-}$ mice. PTH increased both ECaCs mRNA expression in the PRTC of $VDR^{+/+}$ mice. These results suggest that 1α,25$(OH)_2D_3$ directly modulates the gene expression of ECaC1 and ECaC2 together with PTH in the kidney of mice. 1α,25$(OH)_2D_3$ also modulates the gene expression of ECaC2 in the intestine of mice, however, further studies are needed to elucidate the direct action of 1α,25$(OH)_2D_3$ on the expression of ECaC2 in the intestine.

Keywords: Epithelial calcium channels; Gene expression; Intestine and kidney; VDR knockout mice

1. Introduction

Calcium (Ca) transport in the intestine and kidney is stimulated by 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. Ca regulating proteins including calbindin-D, plasma membrane Ca ATPase (PMCA1b) and Na/Ca exchanger (NCX1), are thought to be involved in this process and gene expression of these proteins have been postulated to be regulated by 1α,25$(OH)_2D_3$. Recently, epithelial Ca channels 1 and 2 (ECaC1 and ECaC2) have been identified [1,2] and cloned from several vitamin D-target tissues of several species [3–6] and it is expected that they may serve as a gate-keeper of transepithelial active Ca transport [7]. However, the role of 1α,25$(OH)_2D_3$ in the regulation of ECaC expression remains unclear.

In the present study, we examined the effect of 1α,25$(OH)_2D_3$ on the ECaC mRNA expression in the intestine and kidney of $VDR^{+/+}$ and $VDR^{-/-}$ mice. In addition, mRNA expression of ECaC in the primary renal tubular cells (PRTC) isolated from $VDR^{+/+}$ and $VDR^{-/-}$ mice were also evaluated.

2. Materials and methods

2.1. Animals

C57BL/6J $VDR^{-/-}$ mice were generated by homologous gene targeting as described previously [8]. Null mutant mice were obtained by intercrossing the heterozygous VDR knockout female and male mouse. Mice were weaned at 3 weeks of age and were then fed ad libitum a normal calcium diet for 4 weeks. Age-matched C57BL/6J male mice were used as $VDR^{+/+}$ mice and fed ad libitum a normal Ca diet. Mice were injected intravenously with a single dose of 6.25 μg/kg of 1α,25$(OH)_2D_3$ in the time-course study and a single dose of the increasing amounts (0.1–10 μg/kg) of 1α,25$(OH)_2D_3$ in the dose-dependent study. The intestine and kidney of the mice were collected at the indicated time interval for measuring mRNA levels of ECaC1, ECaC2, calbindin-D_{9k} and β-actin.

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2.2. Primary cell culture

Primary renal tubular cells (PRTC) were isolated from the kidneys of $VDR^{+/+}$ and $VDR^{-/-}$ mice, 7 weeks of age, as described elsewhere. Kidneys were minced and digested in the Krebs–Henseleit buffer containing collagenase type I at 37 °C for 45 min. After centrifugation, cells were suspended in the H-DMEM/Ham F12 (1:1) medium containing 5% FCS and 10^6 cells were seeded in 6-well culture dishes and cultured at 37 °C for 24 h.

2.3. Real-time quantitative PCR for ECaC1, ECaC2 and calbindin-D_{9k}

The RNA purified from the intestine and kidney were reverse- transcribed. Quantitative analysis of gene expression was performed using the GeneAmp 5700 Sequence Detection System (PE Biosystems, Foster City, CA) and the SYBR Green core reagent kit (PE Biosystems, Foster City, CA).

3. Results

As shown in Fig. 1, RT-PCR analysis revealed that in $VDR^{+/+}$ mice, 7 weeks of age, ECaC2 mRNA was confirmed in the intestine and kidney, while ECaC1 mRNA was exclusively confined to the kidney. In a time-course study in $VDR^{+/+}$ mice, intestinal ECaC2 mRNA expression began to increase from 3 h and reached to the maximum at 6 h after injection of 1α,25$(OH)_2D_3$. Similarly, renal ECaC1 and ECaC2 mRNA expression began to increase from 3 h

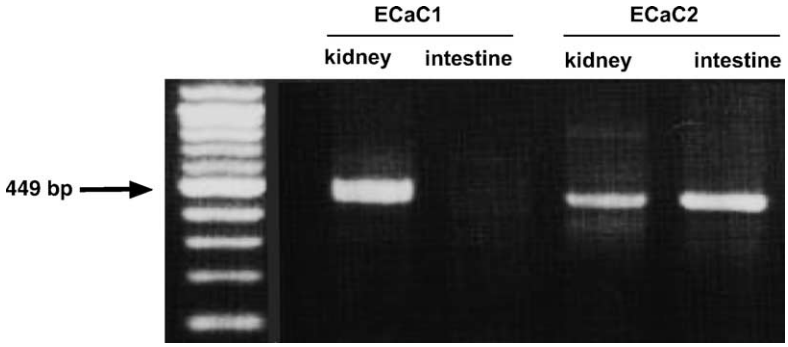

Fig. 1. Detection of the mRNAs of ECaC1 and ECaC2 in the kidney and the intestine of $VDR^{+/+}$ mice by RT-PCR.

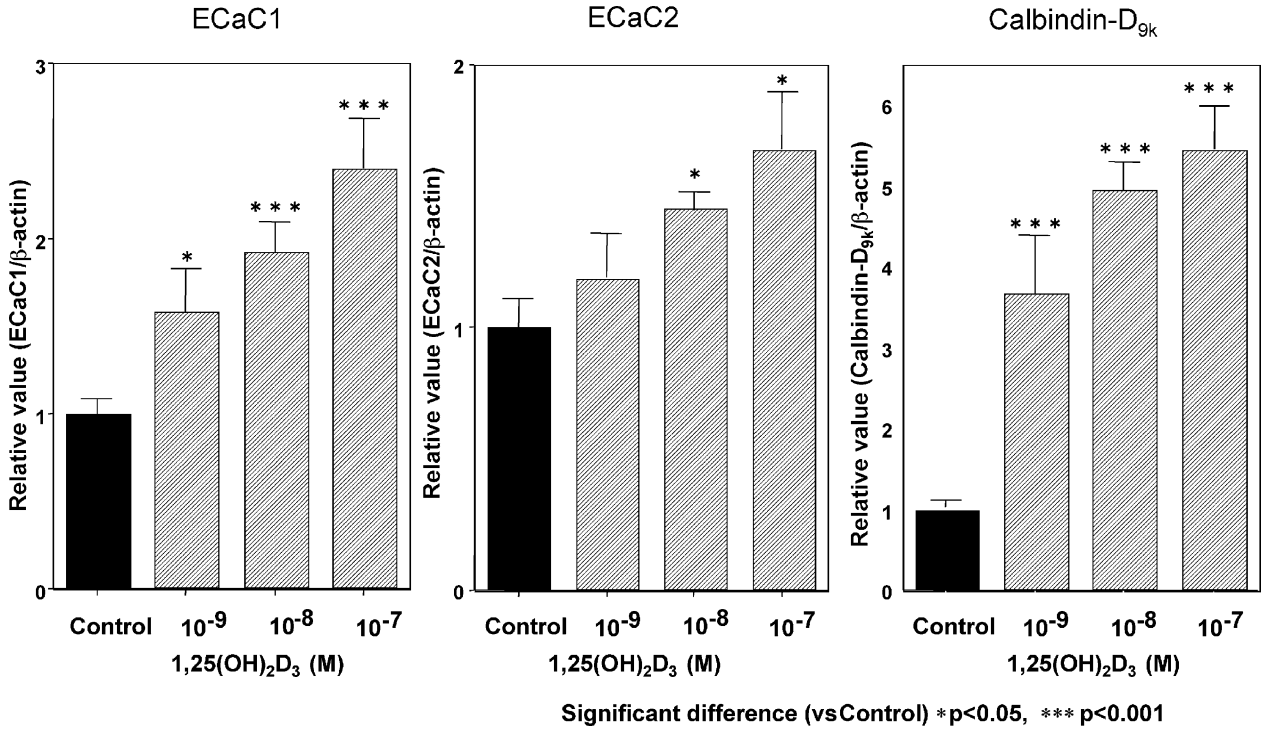

Fig. 2. Dose-dependent changes in the mRNA levels of ECaC1, ECaC2 and calbindin-D_{9k} induced by 1α,25$(OH)_2D_3$ in the PRTC isolated from $VDR^{+/+}$ mice.

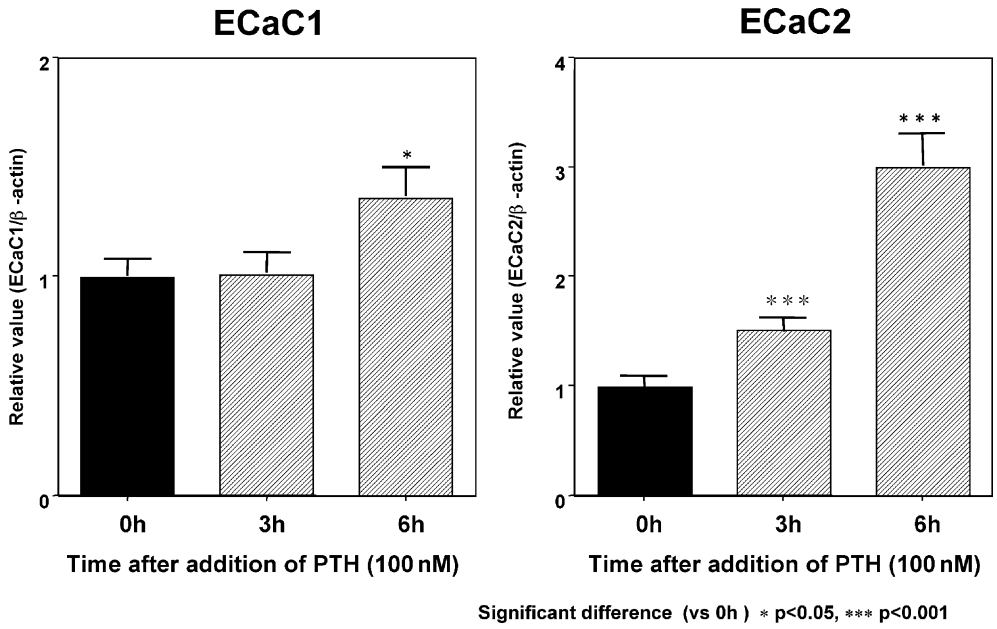

Fig. 3. Time-dependent changes in the mRNA levels of ECaC1 and ECaC2 induced by PTH in the PRTC isolated from $VDR^{+/+}$ mice.

and continuously increased by 9 h. ECaC mRNA expression in the intestine and kidney of $VDR^{+/+}$ mice increased dose-dependently by $1\alpha,25(OH)_2D_3$. The mRNA levels of ECaC2 and calbindin-D_{9k} in the intestine of $VDR^{-/-}$ mice were extremely reduced compared to those of $VDR^{+/+}$ mice, while both mRNA levels of ECaC1 and ECaC2 in the kidney of $VDR^{-/-}$ mice were almost similar to those of $VDR^{+/+}$ mice, although renal calbindin-D_{9k} mRNA levels were remarkably reduced in $VDR^{-/-}$ mice. The PRTC isolated from $VDR^{+/+}$ mice exhibited a dose-dependent response to $1\alpha,25(OH)_2D_3$ treatment in inducing the mRNA expression of ECaC1, ECaC2 and calbindin-D_{9k} (Fig. 2), but not in the PRTC from $VDR^{-/-}$ mice. PTH induced the mRNA expression of ECaC1 and ECaC2 in the PRTC isolated from $VDR^{+/+}$ mice (Fig. 3). These in vivo and in vitro results clearly indicate that ECaC2 expression in the intestine is regulated by $1\alpha,25(OH)_2D_3$ and ECaC1 and ECaC2 expression in the kidney are regulated by not only $1\alpha,25(OH)_2D_3$ but also PTH.

4. Discussion

The present study demonstrates that ECaC2 expression in the intestine and ECaC1 and ECaC2 expression in the kidney of mice are up-regulated by $1\alpha,25(OH)_2D_3$. It is generally accepted that Ca transport in the intestine and kidney is regulated by three steps, the first step in apical membrane including Ca channels (ECaCs), the second step in cytosol including calbindin-D_{9k}, and the third step in basolateral membrane including plasma membrane Ca ATPase ($PMCA_{1b}$) and Na/Ca exchanger (NCX1). These steps are believed to be vitamin D-dependent and the role of $1\alpha,25(OH)_2D_3$ in these processes largely remains unclear. Recently, two epithelial Ca channels ECaC1 and ECaC2 have been cloned from several vitamin D-target tissues of several species and vitamin D response elements (VDREs) were detected in the promoter region of human ECaC1 [4]. Based on these findings, regulation of ECaCs by $1\alpha,25(OH)_2D_3$ has been postulated [9]. However, Weber et al. reported that ECaC expression in the intestine and the kidney was regulated by extracellular calcium, but not by $1\alpha,25(OH)_2D_3$ or estrogen in vivo in mice [6]. Van Cromphaut et al. also reported that ECaC expression in $VDR^{-/-}$ mice was down-regulated in the intestine, but not in the kidney [9]. In the present study, we examined whether $1\alpha,25(OH)_2D_3$ directly regulates ECaC expression in the target tissues of mice in vivo and in vitro. In the in vivo study in $VDR^{+/+}$ mice, $1\alpha,25(OH)_2D_3$ up-regulated the ECaC2 expression in the intestine and both ECaCs expression in the kidney. Intriguingly, the ECaCs mRNA levels in the kidney of $VDR^{-/-}$ mice were almost similar to those in $VDR^{+/+}$ mice. The result suggests that an intrinsic factor(s) except for $1\alpha,25(OH)_2D_3$ may be involved in the regulation of ECaC expression in the kidney. To clarify this possibility, we isolated the PRTC from $VDR^{+/+}$ mice and $VDR^{-/-}$ mice and examined the effects of $1\alpha,25(OH)_2D_3$ and PTH on ECaC expression in the PRTC. The results clearly indicate that $1\alpha,25(OH)_2D_3$ has the direct effect on the ECaC expression and PTH also up-regulates ECaC2 expression directly and more strongly than ECaC1 expression. The direct action of $1\alpha,25(OH)_2D_3$ on the ECaC2 expression in the intestine is currently under investigation using an isolated duodenum cell culture system in our laboratory.

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