JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2011, 62, 5, 499-504
www.jpp.krakow.pl

Review article

K.C. CHOI, B.S. AN, H. YANG, E.B. JEUNG

REGULATION AND MOLECULAR MECHANISMS OF CALCIUM TRANSPORT GENES: DO THEY PLAY A ROLE IN CALCIUM TRANSPORT IN THE UTERINE ENDOMETRIUM?

Laboratory of Veterinary Biochemistry, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea

Maintenance of calcium (Ca) balance in the uterus is critically important for many physiological functions, including smooth muscle contraction during embryo implantation. Ca transport genes, *i.e*., transient receptor potential cation channel subfamily V members 5/6 (TRPV5/6), calbindins, plasma membrane Ca^{2+}-ATPase 1 (PMCA1), and NCX1/NCKX3, may play roles in the uterus for Ca transport and reproductive function. Although these Ca transport genes may have a role in Ca metabolism, their role(s) and molecular mechanisms require further elucidation. In this review, we highlight the expression and regulation of Ca transport genes in the uterus to clarify their potential role(s). Since Ca transport genes are abundantly expressed in reproductive tissues in a distinct manner, they may be involved in specific uterine functions including fetal implantation, Ca homeostasis, and endometrial cell production.

Key words: *calbindins, endometrium, transient receptor potential cation channel subfamily V members 6 (TRPV6), plasma membrane Ca^{2+}-ATPase 1 (PMCA1), NCX1/NCKX3*

INTRODUCTION

Calcium (Ca) is a vital mineral for *in vivo* survival and homeostasis. A great deal of progress has been made in determining how Ca metabolism is involved in regulating physiological functions; however, the process remains unresolved. In the regulation of calcium ions (Ca^{2+}) for *in vivo* functions, active Ca transport proteins play critical roles in three functional steps: 1) Ca influx, 2) transfer through the cytosol, and 3) extrusion into the blood-stream, which in turn are mediated at the cellular level by three types of proteins: 1) Ca entry-channel proteins of the outer membrane, 2) cytosolic buffering or transfer proteins, and 3) excretory pump proteins (1).

The main Ca entry channels include two highly selective Ca channels on the apical plasma membrane, transient receptor potential cation channel subfamily V members 6 and 5 (TRPV6 and TRPV5) (1). In addition, intracellular Ca^{2+}-binding proteins, *i.e*., two calbindins, calbindin-D9k (CaBP-9k) and -D28k (CaBP-28k), may participate in shuttling Ca^{2+} from the apical to the basolateral membrane (2-5), while the sodium-calcium (Na^{+}/Ca^{2+}) exchanger (NCX1) and plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) play an essential role in extruding Ca^{2+} (2, 6).

These Ca transport proteins have been shown to be expressed in diverse cells and tissues in our body. To understand their roles in Ca homeostasis, we have focused on the expression and regulation of these Ca transport proteins in various tissues including the uterus, kidney, and duodenum of animal models and humans for a decade. In this review, we described the regulation of Ca transport proteins and their potential role(s) in reproductive tissue (*i.e*., endometrium), for implantation and pregnancy.

TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL SUBFAMILY V MEMBERS 5 AND 6 (TRPV5 AND 6)

In mammals, Ca^{2+} appear to play a critical role in controlling uterine muscle contraction and embryo implantation. The balance between contraction and relaxation is extremely important throughout pregnancy and during labor. However, regulation of uterine Ca is not yet fully understood. A model for Ca^{2+} in intestinal cells suggests that Ca flows into the cytoplasm *via* channel proteins, *i.e*. Ca transporters. These transporters transfer Ca using Ca-binding proteins (Calbindin-D9k or -28k), which are extruded from the cell membrane by plasma membrane Ca^{2+} ATPase (1). TRPV5 and TRPV6 are found in the apical membranes of intestinal and renal epithelial cells, and have been proposed as mediators of Ca uptake during trans-cellular transport (7). TRPV6 is known as epithelial Ca channel 2 (EcaC2) and Ca transporter 1 (CaT1), while TRPV5 is also referred to as EcaC1 and CaT2. These closely-related proteins are expressed primarily in cells of the duodenum and kidney that are involved in Ca absorption or re-absorption (1).

TRPV6 was first cloned from rat duodenum and was detected subsequently in both human and mouse duodenum (8-10). The genes encoding TRPV5 and TRPV6 proteins are located closely together on the same chromosome and their genomic structures are similar (8). TRPV6 is expressed in the duodenum, jejunum, ileum, kidney and exocrine tissues such as the pancreas, prostate, and mammary and sweat glands (1, 7, 8). In contrast, TRPV5 is expressed in the kidney and human syncytiotrophoblasts (11).

In the reproductive organs, *TRPV6* is expressed in the placenta as well as in the uterus (12). Placental TRPV6 plays a

role in Ca transport to the fetus (13). Duodenal and renal TRPV6 expression is regulated by vitamin D, estrogen and dietary Ca. An active form of vitamin D increases duodenal Ca absorption, and abnormal Ca absorption has been observed in vitamin D receptor-knockout mice (14, 15). Dietary Ca can also induce duodenal and renal *TRPV6* mRNA expression (4, 14) and estrogen therapy in menopausal women induces duodenal *TRPV6* mRNA, suggesting that this hormone independently modulates TRPV6 expression (5, 15).

In our previous study, uterine TRPV6 expression in rats was found to be similar to that of CaBP-9k expression in mice (16). In addition, we previously examined the expression and regulation of *TRPV6* in the (17) uterus of mice during the estrous cycle and pregnancy to elucidate the functional relationships between these two uterine Ca-processing genes (16). Expression of *TRPV6* mRNA in the uterus varied during the estrous cycle (pro-, di-, and estrus). Expression of the less abundant *TRPV5* mRNA was not determined in our previous study. Consistently, Weber *et al*. suggested that *TRPV5* is expressed only in the kidney (8). The *TRPV6* transcript was highly expressed at estrus, an E2-dominant phase in the reproductive cycle, implying that it might be involved in a specific uterine function such as Ca^{2+} transport (16). In our previous study, rat *TRPV6* transcripts in the uterus were highly expressed at diestrus (12). Taken together, these results indicate that uterine *TRPV6* is regulated differently during the estrous cycle in rats and mice.

In addition, the expression of TRPV6 mRNA and protein in human endometrial tissues was demonstrated for the first time, and the expression of this protein was shown to be induced by E2 during the proliferative phases of the menstrual cycle (17). Endometrial TRPV6 was abundantly expressed in the endometrial epithelial layer and glandular epithelial cells, indicating that TRPV6 may be involved in uterine functions, including fetal implantation, Ca homeostasis, and endometrial cell production in the human reproductive system (17).

CALBINDINS

A number of Ca-regulating genes are involved in uterine function during the estrous cycle and pregnancy (18). During the window of receptivity of the uterine endometrium, Ca-binding proteins (CaBP-9k and -28k), monoclonal non-specific suppressor factor beta, and splicing factor SC35 are involved in embryo implantation (19-21). In addition, there is evidence that CaBP-9k and -28k are required during the early phase of embryo implantation, suggesting that the regulation of Ca availability in the vicinity of the implanting embryo is critical for successful attachment (22, 23). In the duodenum, CaBP-9k and TRPV6 were shown to have similar expression patterns; however, uterine CaBP-9k is regulated by different hormones and expressed at different stages of the estrous cycle (24, 25). The expression of CaBP-9k is increased by E2 and at estrus in rats but its expression pattern is controlled by P4 and unaffected at diestrus in mice (26, 27).

As previously shown, the initial up-regulation of CaBP-28k increases the storage capacity for Ca^{2+} in luminal epithelial cells, and its subsequent specific down-regulation leads to an increase in free Ca^{2+} concentration (22). In mammalian enterocytes, free Ca^{2+} are bound to cytosolic CaBP-9k and transferred across the cells by facilitated diffusion (28). This transport of Ca^{2+} by CaBP-9k helps to maintain homeostasis by keeping intracellular Ca^{2+} concentrations below 10^{-7}M, preventing premature cell death by apoptosis. Thus, it can be speculated that the role of CaBP-28k protein in the uterine luminal epithelium is also to enhance Ca^{2+} uptake by increasing the cell-buffering capacity and stimulating the Ca entry mechanism in these cells. The excess Ca result from a down-regulation of CaBP-28k may trigger apoptosis in these specific epithelial cells because high concentrations of free Ca^{2+} are reported to cause apoptosis in many different cell types (29) and CaBP-28k is able to inhibit apoptosis in osteoblastic cells (30). This apoptosis could, in turn, destabilize the epithelial barrier at the implantation site and facilitate trophoblast invasion and implantation.

We previously determined that Ca-related proteins are regulated by sex-steroid hormones in the uterus of rodents (12, 16, 31, 32). It is well known that the status of the uterine cavity is important for successful implantation and is influenced by the secretory activity of the glandular epithelium (19, 33). In our current study, CaBP-28k mRNA and protein were expressed in human endometrial tissues (34). The levels of Ca-related proteins fluctuated in E2-predominant stages (16, 27, 31). CaBP-28k mRNA and protein were expressed at these stages (proliferative (early, mid, late) and secretory (only early) phase), followed by a decline in the secretory phase (mid-, late-) in the human endometrium, as demonstrated in this study. This result is in agreement with the pattern of CaBP-28k expression in the uterus of both mice and humans (22). Therefore, these results indicate that this parallel pattern of CaBP-28k expression may also be involved in fetal implantation in humans (34).

PLASMA MEMBRANE CA^{2+} ATPASE 1

Plasma membrane Ca^{2+} ATPase 1 (PMCA1) is an ATP-dependent transporter that pumps Ca out of the cytosol. PMCA1 was first detected in erythrocyte membranes and found to have a high affinity for Ca^{2+} (35). In many species, PMCA1 is involved in Ca homeostasis (35-37). There are four PMCA isoforms (PMCA1 to 4) that are further divided into several subtypes by alternative splicing. PMCA1 is known as the housekeeping isoform because its mRNA is found in all tissues. However, PMCA1 expression is upregulated in uterine smooth muscle during labor (38), and placental PMCA1 acts as a Ca transporter together with TRPV6. Although a correlation between the regulation of Ca^{2+} homeostasis by TRPV6 and PMCA1 and duodenal and renal function has not yet been established, recent reports have described a role for TRPV6 and PMCA1 in the uterus, duodenum, kidney, and brain (9, 10, 12, 36, 38). We have demonstrated the expression of TRPV6 in reproductive organs, including the uterus (12, 16), but its role in this particular organ has not been fully characterized. The importance of maintaining the Ca balance for different uterine functions, including smooth muscle contraction during embryo implantation (19, 21), implies that PMCA1 may play functionally important roles in female reproductive organs. In our previous study, endometrial PMCA1 was abundantly expressed in the endometrial epithelial layer and glandular epithelial cells, indicating that PMCA1 may be involved in uterine functions, including fetal implantation, Ca homeostasis, and endometrial cell production in the human reproductive system (17).

NCX1/NCKX3 EXCHANGERS

Plasma membrane Na^{+}/Ca^{2+} exchangers are also important components of intracellular Ca homeostasis and electrical conduction. A member of the family of potassium (K)-dependent Na^{+}/Ca^{2+}exchangers, NCKX3 plays a critical role in the transport of intracellular Ca and one potassium ion (K^{+}) in exchange for four extracellular Na^{+}. *NCKX3* mRNA transcripts are abundant in brain and smooth muscle, and many other tissues have been shown to express NCKX3 at relatively lower levels, including uterus, aorta, and intestine (39). Plasma membrane

Na^+/Ca^{2+} exchange proteins in mammals have been divided into two families, one in which Ca^{2+} flux is dependent only on Na (NCX1-3), and the other in which Ca^{2+} flux is also dependent on K (NCKX1-6) (39-41). For both NCX and NCKX exchangers, forward-mode and reverse-mode exchanges are possible. In cells or tissues, Na^+/Ca^{2+} (and K^+) gradients localize to the membrane, thus, exchangers transport across a membrane potential (42, 43).

NCX1 is abundantly expressed in heart, brain, kidney and smooth muscle (44), while expression of NCX2 and NCX3 is limited to brain and skeletal muscle (42, 44, 45). NCKX1 is expressed only in retinal rod photoreceptors, whereas NCKX2 is restricted to brain neurons and cone photoreceptors (42). NCKX3 and NCKX4 are expressed in brain, as well as aorta, uterus and intestine, all of which are rich in smooth muscle cells (39, 46, 47). Recently, it was demonstrated that NCKX5 is expressed in skin and retinal pigmented epithelium, where it is thought to localize to the melanosomal membrane, not the plasma membrane (48). The physiological function of NCKX6 was recently reported; however, the data is somewhat controversial (47, 49).

While a physiological role for NCKX and NCX proteins in vascular contraction through the regulation of Ca^{2+} homeostasis has not yet been definitively established, recent reports have described NCKX and NCX functions in brain, spermatozoa, mast cells and platelets (50-54). NCKX3 is expressed in reproductive organs, including the uterus (39); however, the role of NCKX3 in the uterus has yet to be fully characterized.

As the major sex steroid hormones, E2 and P4 can induce changes in the structure and function of the uterus and regulate estrous cycle progression. To determine the effect of these hormones on uterine NCKX3 expression, we injected immature mice daily with E2 and/or P4. Treatment with E2 and/or P4 resulted in the suppression of uterine expression of NCKX3 at the mRNA and protein levels, although the effect of combined treatment with E2 and P4 was not synergistic, suggesting that E2 or P4 functions as an inhibitory regulator of uterine NCKX3 transcription in mice (31). In addition, we analyzed the localization of NCKX3 protein during the estrous cycle and the effect of endogenous sex steroid hormones. Immunohistochemistry revealed that it was mainly localized in the endothelial layers and glands in the uterus of mature female rats during proestrus (55). This is the first demonstration of the expression of NCKX3 mRNA and protein in the uterus of rats, and our analysis showed that its expression level fluctuated during the estrous cycle in female rats, indicating that uterine NCKX3 may play an important role in reproductive system functions in female rats (55).

Immunohistochemical studies showed that the NCKX3 protein was highly expressed in the cytoplasm of endometrial epithelial cells during the early-proliferative phase. Also, NCKX3 was weakly expressed in endometrial epithelial cells compared to glandular epithelial cells during the early-secretory phase. However, NCX1 was abundantly expressed in the cytoplasm of both endometrial and glandular epithelial cells in the proliferative and secretory phases. In previous studies, on the day of implantation (day 4-5), mouse blastocysts show weak staining for genes expressed in the endometrial layer (in both the inner cell mass and trophectoderm) (22, 56). The S100 Ca binding protein A4 (S100A4) is involved in regulating the

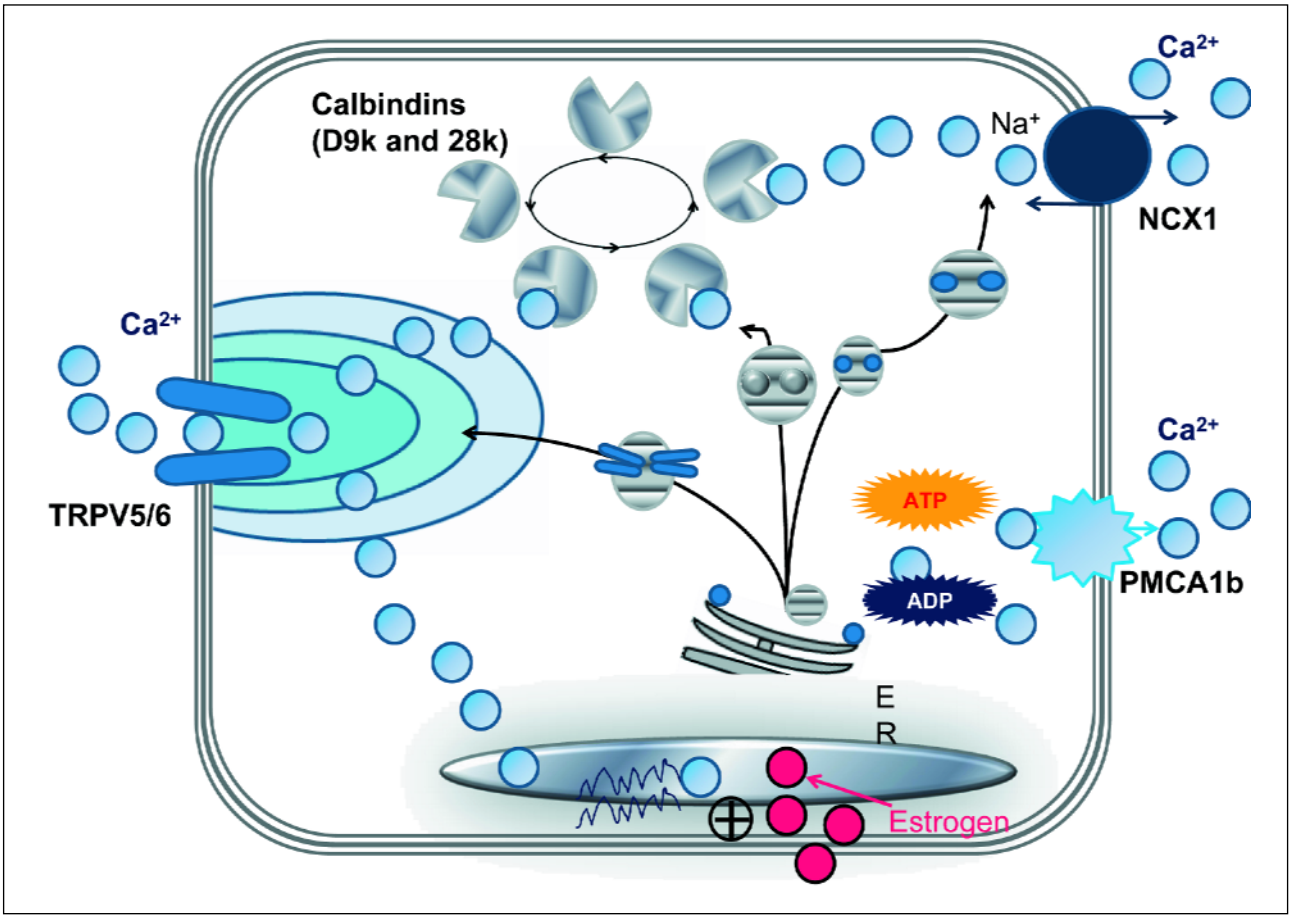

Fig. 1. Potential role(s) and regulation of calcium (Ca) transport genes, *i.e.*, TRPV5/6, calbindins, PCMA1, and NCX1/NCKX3, in reproductive tissues for Ca transport and reproductive functions.

organization of the actin cytoskeleton (57, 58). In several studies, Ca-related genes were abundantly expressed in the endometrial layer pre-implantation; however, uterine Ca-related proteins levels were lower in the endometrial layers than during the implantation period (22, 56). It remains to be established whether the weaker expression of NCKX3 seen in epithelial cells during the early-secretory phase reflects decreased NCKX3 translation and whether steroid hormones are involved in these effects. Ca-related proteins, which are crucial for early implantation, are also expressed in the endometrial layer (19, 21, 22). Therefore, uterine NCKX3 may have two possible functions, either in embryo implantation, or as a Ca regulator necessary for the secretion of related substances into the uterine lumen. Recent studies examining the physiological role(s) of NCKX3 were restricted to the contraction of vascular smooth muscle cells and NCKX function in the brain, spermatozoa, mast cells, and platelets (39, 40, 42, 44, 50).

In our previous study, we analyzed localization of the NCKX3 protein during the menstrual cycle and the effect of endogenous sex-steroid hormones on the regulation of NCKX3 (59). Immunohistochemistry revealed that NCKX3 was mainly localized in the endothelial layers and glandular epithelial cells of the human endometrium, and its level varied throughout the menstrual cycle. These results indicate that NCKX3 is abundantly expressed within the human endometrium at both transcriptional and translational levels, and these level appears to be regulated by a steroid hormone, in particular, E2 during the human menstrual cycle (59).

CONCLUSION

The homeostasis of intracellular Ca is critical to maintain our body. The contraction and relaxation of smooth muscle tissues including cardiovascular tissues, urinary bladder, uterus, gastrointestinal tract, respiratory tract, and male and female reproductive tissues are under the control of intracellular Ca concentration (60, 61). Disturbance of homeostasis of Ca has been known to cause serious diseases such as diabetes, mineral-bone disorder and chronic kidney disease (62, 63). Maintenance of Ca balance in the uterus is also critically important for many physiological functions, including smooth muscle contraction during embryo implantation. Based on previous studies by ourselves and others, potential role(s) and regulation of Ca transport genes, *i.e*., TRPV5/6, calbindins, PMCA1, and NCX1/NCKX3, are hypothesized in the reproductive tissues for Ca transport and their functions (*Fig. 1*). Although these Ca transport genes may play a role in Ca metabolism in these reproductive tissues, their role(s) and molecular mechanisms warrant further elucidation. It is definite that these Ca transport genes are expressed at the transcriptional and translational levels in rodent and human endometrial tissues, and the expression of these proteins appears to be induced by steroids, *i.e*., E2 and P4, during the proliferative phases of the estrous and menstrual cycle. Since Ca transport genes are abundantly expressed in the reproductive tissues in a distinct manner, they may be involved in specific uterine functions, including fetal implantation, Ca homeostasis, and endometrial cell production in the reproductive system.

Acknowledgements: This work was actively supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government to KCC (no. 2011-0015385) and to EBJ (No. 2010-0011433). In addition, the authors appreciate Ms. Nam-Hee Kang for assistance to make a figure.

Conflict of interests: None declared.

REFERENCES

1. Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. *Physiol Rev* 2005; 85: 373-422.
2. Choi KC, Jeung EB. Molecular mechanism of regulation of the calcium-binding protein calbindin-D(9k), and its physiological role(s) in mammals: a review of current research. *J Cell Mol Med* 2008; 12: 409-420.
3. Diepens RJ, den Dekker E, Bens M, *et al*. Characterization of a murine renal distal convoluted tubule cell line for the study of transcellular calcium transport. *Am J Physiol Renal Physiol* 2004; 286: F483-F489.
4. Hoenderop JG, Nilius B, Bindels RJ. Molecular mechanism of active Ca^{2+} reabsorption in the distal nephron. *Annu Rev Physiol* 2002; 64: 529-549.
5. Van Cromphaut SJ, Rummens K, Stockmans I, *et al*. Intestinal calcium transporter genes are upregulated by estrogens and the reproductive cycle through vitamin D receptor-independent mechanisms. *J Bone Miner Res* 2003; 18: 1725-1736.
6. Christakos S, Dhawan P, Benn B, *et al*. Vitamin D: molecular mechanism of action. *Ann NY Acad Sci* 2007; 1116: 340-348.
7. den Dekker E, Hoenderop JG, Nilius B, Bindels RJ. The epithelial calcium channels, TRPV5 & TRPV6: from identification towards regulation. *Cell Calcium* 2003; 33: 497-507.
8. Weber K, Erben RG, Rump A, Adamski J. Gene structure and regulation of the murine epithelial calcium channels ECaC1 and 2. *Biochem Biophys Res Commun* 2001; 289: 1287-1294.
9. Barley NF, Howard A, O'Callaghan D, Legon S, Walters JR. Epithelial calcium transporter expression in human duodenum. *Am J Physiol Gastrointest Liver Physiol* 2001; 280: G285-G290.
10. Peng JB, Chen XZ, Berger UV, *et al*. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J Biol Chem* 1999; 274: 22739-22746.
11. Bernucci L, Henriquez M, Diaz P, Riquelme G. Diverse calcium channel types are present in the human placental syncytiotrophoblast basal membrane. *Placenta* 2006; 27: 1082-1095.
12. Kim HJ, Lee GS, Ji YK, Choi KC, Jeung EB. Differential expression of uterine calcium transporter 1 and plasma membrane Ca^{2+} ATPase 1b during rat estrous cycle. *Am J Physiol Endocrinol Metab* 2006; 291: E234-E241.
13. Suzuki Y, Kovacs CS, Takanaga H, Peng JB, Landowski CP, Hediger MA. Calcium channel TRPV6 is involved in murine maternal-fetal calcium transport. *J Bone Miner Res* 2008; 23: 1249-1256.
14. Van Cromphaut SJ, Dewerchin M, Hoenderop JG, *et al*. Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects. *Proc Natl Acad Sci USA* 2001; 98: 13324-13329.
15. Van Abel M, Hoenderop JG, Dardenne O, *et al*. 1,25-dihydroxyvitamin D(3)-independent stimulatory effect of estrogen on the expression of ECaC1 in the kidney. *J Am Soc Nephrol* 2002; 13: 2102-2109.
16. Lee GS, Jeung EB. Uterine TRPV6 expression during the estrous cycle and pregnancy in a mouse model. *Am J Physiol Endocrinol Metab* 2007; 293: E132-E138.
17. Yang H, Choi KC, Hyun SH, Jeung EB. Coexpression and estrogen-mediated regulation of TRPV6 and PMCA1 in the human endometrium during the menstrual cycle. *Mol Reprod Dev* 2011; 78: 274-282.
18. Daston GP, Naciff JM. Gene expression changes related to growth and differentiation in the fetal and juvenile reproductive system of the female rat: evaluation of microarray results. *Reprod Toxicol* 2005; 19: 381-394.

19. Salamonsen LA, Nie G, Dimitriadis E, Robb L, Findlay JK. Genes involved in implantation. *Reprod Fertil* Dev 2001; 13: 41-49.
20. Simmen RC, Simmen FA. Progesterone receptors and Sp/Kruppel-like family members in the uterine endometrium. *Front Biosci* 2002; 7: d1556-d1565.
21. Luu KC, Nie GY, Hampton A, Fu GQ, Liu YX, Salamonsen LA. Endometrial expression of calbindin (CaBP)-d28k but not CaBP-d9k in primates implies evolutionary changes and functional redundancy of calbindins at implantation. *Reproduction* 2004; 128: 433-441.
22. Luu KC, Nie GY, Salamonsen LA. Endometrial calbindins are critical for embryo implantation: evidence from in vivo use of morpholino antisense oligonucleotides. *Proc Natl Acad Sci USA* 2004; 101: 8028-8033.
23. Mathieu CL, Burnett SH, Mills SE, Overpeck JG, Bruns DE, Bruns ME. Gestational changes in calbindin-D9k in rat uterus, yolk sac, and placenta: implications for maternal-fetal calcium transport and uterine muscle function. *Proc Natl Acad Sci USA* 1989; 86: 3433-3437.
24. Song Y, Peng X, Porta A, *et al*. Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1,25 dihydroxyvitamin D3 in the intestine and kidney of mice. *Endocrinology* 2003; 144: 3885-3894.
25. Bronner F. Mechanisms of intestinal calcium absorption. *J Cell Biochem* 2003; 88: 387-393.
26. An BS, Choi KC, Hong EJ, Jung YW, Manabe N, Jeung EB. Differential transcriptional and translational regulations of calbindin-D9k by steroid hormones and their receptors in the uterus of immature mice. *J Reprod Dev* 2004; 50: 445-453.
27. Krisinger J, Dann JL, Currie WD, Jeung EB, Leung PC. Calbindin-D9k mRNA is tightly regulated during the estrous cycle in the rat uterus. *Mol Cell Endocrinol* 1992; 86: 119-123.
28. Walters JR. Calbindin-D9k stimulates the calcium pump in rat enterocyte basolateral membranes. *Am J Physiol* 1989; 256: G124-G128.
29. Tombal B, Denmeade SR, Gillis JM, Isaacs JT. A supramicromolar elevation of intracellular free calcium ([Ca(2+)](i)) is consistently required to induce the execution phase of apoptosis. *Cell Death Differ* 2002; 9: 561-573.
30. Bellido T, Huening M, Raval-Pandya M, Manolagas SC, Christakos S. Calbindin-D28k is expressed in osteoblastic cells and suppresses their apoptosis by inhibiting caspase-3 activity. *J Biol Chem* 2000; 275: 26328-26332.
31. Yang H, Lee GS, Yoo YM, Choi KC, Jeung EB. Sodium/potassium/calcium exchanger 3 is regulated by the steroid hormones estrogen and progesterone in the uterus of mice during the estrous cycle. *Biochem Biophys Res Commun* 2009; 385: 279-283.
32. Hong EJ, Choi KC, Jeung EB. Induction of calbindin-D9k messenger RNA and protein by maternal exposure to alkylphenols during late pregnancy in maternal and neonatal uteri of rats. *Biol Reprod* 2004; 71: 669-675.
33. Cai L, Zhang J, Duan E. Dynamic distribution of epidermal growth factor during mouse embryo peri-implantation. *Cytokine* 2003; 23: 170-178.
34. Yang H, Kim TH, Lee HH, *et al*. Expression of calbindin-D28k and its regulation by estrogen in the human endometrium during the menstrual cycle. *Reprod Biol Endocrinol* 2011; 9: 28.
35. Schatzmann HJ. ATP-dependent Ca^{++}-extrusion from human red cells. *Experientia* 1966; 22: 364-365.
36. Stauffer TP, Guerini D, Celio MR, Carafoli E. Immunolocalization of the plasma membrane Ca2+ pump isoforms in the rat brain. *Brain Res* 1997; 748: 21-29.
37. Shull GE, Greeb J. Molecular cloning of two isoforms of the plasma membrane Ca^{2+}-transporting ATPase from rat brain. Structural and functional domains exhibit similarity to Na+,K+- and other cation transport ATPases. *J Biol Chem* 1988; 263: 8646-8657.
38. Tribe RM, Moriarty P, Poston L. Calcium homeostatic pathways change with gestation in human myometrium. *Biol Reprod* 2000; 63: 748-755.
39. Kraev A, Quednau BD, Leach S, *et al*. Molecular cloning of a third member of the potassium-dependent sodium-calcium exchanger gene family, NCKX3. *J Biol Chem* 2001; 276: 23161-23172.
40. Dong H, Jiang Y, Triggle CR, Li X, Lytton J. Novel role for K^+-dependent Na^+/Ca^{2+} exchangers in regulation of cytoplasmic free Ca^{2+} and contractility in arterial smooth muscle. *Am J Physiol Heart Circ Physiol* 2006; 291: H1226-H1235.
41. Tsoi M, Rhee KH, Bungard D, *et al*. Molecular cloning of a novel potassium-dependent sodium-calcium exchanger from rat brain. *J Biol Chem* 1998; 273: 4155-4162.
42. Lytton J, Li XF, Dong H, Kraev A. K^+-dependent Na^+/Ca^{2+} exchangers in the brain. *Ann NY Acad Sci* 2002; 976: 382-393.
43. Blaustein MP, Lederer WJ. Sodium/calcium exchange: its physiological implications. *Physiol Rev* 1999; 79: 763-854.
44. Nicoll DA, Longoni S, Philipson KD. Molecular cloning and functional expression of the cardiac sarcolemmal Na(+)-Ca^{2+} exchanger. *Science* 1990; 250: 562-565.
45. Nicoll DA, Quednau BD, Qui Z, Xia YR, Lusis AJ, Philipson KD. Cloning of a third mammalian Na^+-Ca^{2+} exchanger, NCX3. *J Biol Chem* 1996; 271: 24914-24921.
46. Li XF, Kraev AS, Lytton J. Molecular cloning of a fourth member of the potassium-dependent sodium-calcium exchanger gene family, NCKX4. *J Biol Chem* 2002; 277: 48410-48417.
47. Cai X, Lytton J. Molecular cloning of a sixth member of the K^+-dependent Na^+/Ca^{2+} exchanger gene family, NCKX6. *J Biol Chem* 2004; 279: 5867-5876.
48. Lamason RL, Mohideen MA, Mest JR, *et al*. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* 2005; 310: 1782-1786.
49. Palty R, Ohana E, Hershfinkel M, *et al*. Lithium-calcium exchange is mediated by a distinct potassium-independent sodium-calcium exchanger. *J Biol Chem* 2004; 279: 25234-25240.
50. Aneiros E, Philipp S, Lis A, Freichel M, Cavalie A. Modulation of Ca^{2+} signaling by Na^+/Ca^{2+} exchangers in mast cells. *J Immunol* 2005; 174: 119-130.
51. Kiedrowski L. High activity of K^+-dependent plasmalemmal Na^+/Ca^{2+} exchangers in hippocampal CA1 neurons. *Neuroreport* 2004; 15: 2113-2116.
52. Kiedrowski L, Czyz A, Baranauskas G, Li XF, Lytton J. Differential contribution of plasmalemmal Na/Ca exchange isoforms to sodium-dependent calcium influx and NMDA excitotoxicity in depolarized neurons. *J Neurochem* 2004; 90: 117-128.
53. Kim MH, Korogod N, Schneggenburger R, Ho WK, Lee SH. Interplay between Na^+/Ca^{2+} exchangers and mitochondria in Ca^{2+} clearance at the calyx of Held. *J Neurosci* 2005; 25: 6057-6065.
54. Kip SN, Gray NW, Burette A, Canbay A, Weinberg RJ, Strehler EE. Changes in the expression of plasma membrane calcium extrusion systems during the maturation of hippocampal neurons. *Hippocampus* 2006; 16: 20-34.
55. Yang H, Yoo YM, Jung EM, Choi KC, Jeung EB. Uterine expression of sodium/potassium/calcium exchanger 3 and its regulation by sex-steroid hormones during the estrous cycle of rats. *Mol Reprod Dev* 2011; 77: 971-977.

56. Surveyor GA, Wilson AK, Brigstock DR. Localization of connective tissue growth factor during the period of embryo implantation in the mouse. *Biol Reprod* 1998; 59: 1207-1213.
57. Brunton VG, MacPherson IR, Frame MC. Cell adhesion receptors, tyrosine kinases and actin modulators: a complex three-way circuitry. *Biochim Biophys Acta* 2004; 1692: 121-144.
58. Kim EJ, Helfman DM. Characterization of the metastasis-associated protein, S100A4. Roles of calcium binding and dimerization in cellular localization and interaction with myosin. *J Biol Chem* 2003; 278: 30063-30073.
59. Yang H, Kim TH, Lee HH, Choi KC, Jeung EB. Distinct expression of the calcium exchangers, NCKX3 and NCX1, and their regulation by steroid in the human endometrium during the menstrual cycle. *Reprod Sci* 2011; 18: 577-585.
60. Guibert C, Ducret T, Savineau JP. Expression and physiological roles of TRP channels in smooth muscle cells. *Adv Exp Med Biol* 2011; 704: 687-706.
61. Martin-Cano FE, Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Spontaneous calcium oscillations in urinary bladder smooth muscle cells. *J Physiol Pharmacol* 2009; 60: 93-99.
62. Demkow U, Winklewski P, Potapinska O, Popko K, Lipinska A, Wasik M. Kinetics of calcium ion concentration accompanying transduction of signals into neutrophils from diabetic patients and its modification by insulin. *J Physiol Pharmacol* 2009; 60(Suppl 5): 37-40.
63. Peacock M. Calcium metabolism in health and disease. *Clin J Am Soc Nephrol* 2010; 5(Suppl 1): S23-S30.
64. Lee GS, Kim HJ, Jung YW, Choi KC, Jeung EB. Estrogen receptor alpha pathway is involved in the regulation of Calbindin-D9k in the uterus of immature rats. *Toxicol Sci* 2005; 84: 270-277.

Received: August 15, 2011
Accepted: September 26, 2011

Author's address: Dr. Eui-Bae Jeung, Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, 361-763, Republic of Korea; Phone: +82-43-261-2397; Fax: +82-43-267-3150; E-mail: ebjeung@chungbuk.ac.kr