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Expression and Regulation of Sodium/Calcium Exchangers, NCX and NCKX, in Reproductive Tissues: Do They Play a Critical Role in Calcium Transport for Reproduction and Development?

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Abstract

Plasma membrane sodium/calcium (Na^+/Ca^{2+}) exchangers are an important component of intracellular calcium $[Ca^{2+}]_i$ homeostasis and electrical conduction. Na^+/Ca^{2+} exchangers, NCX and NCKX, play a critical role in the transport of one $[Ca^{2+}]_i$ and potassium ion across the cell membrane in exchange for four extracellular sodium ions $[Na^+]_e$. Mammalian plasma membrane Na^+/Ca^{2+} exchange proteins are divided into two families: one in which Ca^{2+} flux is dependent only on sodium (NCX1–3) and another in which Ca^{2+} flux is also dependent on potassium (NCKX1–4). Both molecules are capable of forward- and reverse-mode exchange. In cells and tissues, Na^+/Ca^{2+} (and K^+) gradients localize to the cell membrane; thus, the exchangers transport ions across a membrane potential. Uterine NCKX3 has been shown to be involved in the regulation of endometrial receptivity by $[Ca^{2+}]_i$. In the uterus and placenta, NCKX3 expression is regulated by the sex steroid hormone estrogen (E2) and hypoxia stress, respectively. In this chapter, we described the expression and regulation of these proteins for reproductive functions in various tissues including uterus, placenta, and kidney of humans and rodents. Evidence to date suggests that NCKX3 and NCX1 may be regulated in a tissue-specific manner. In addition, we focused on the molecular mechanism involved in the regulation of NCKX3 and NCX1 in mammals, based upon our recent results and those of others.

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10.1 Introduction

The actions of calcium ions (Ca^{2+}) in female reproductive organs have been widely studied for several decades. It has been suggested that Ca^{2+} are involved in uterine smooth muscle contraction, fetal implantation, and fetal bone mineralization. The balance between uterine muscle contraction and relaxation is extremely important throughout pregnancy and during labor. A model of Ca^{2+} transport suggests that calcium flows into the cytoplasm via channel proteins. Two pathways are responsible for Ca^{2+} entry into the body (Peng et al. 2003). In the paracellular pathway, occludin and junction adherence molecular A (JAM-A), which construct tight junctions and regulate paracellular transport, are needed for Ca^{2+} absorption. Paracellular Ca^{2+} absorption is highest in the ileum, while dietary Ca^{2+} absorption via transcellular mechanisms occurs predominantly in the duodenum (Khanal and Nemere 2008). Transepithelial Ca^{2+} active transport occurs according to a well-controlled sequence of events consisting of apical Ca^{2+} entry involving channels such as voltage-dependent Ca^{2+} (VDCCs) and the transient receptor potential (TRP) family, TRPV5 and TRPV6 in particular, followed by the use of calcium-binding proteins (Calbindin-D9k or -28 k) and transient receptor potential vanilloid type 5 and 6 (TRPV5 and TRPV6), which are extruded from the cell membrane by plasma membrane Ca^{2+} ATPase and to a lesser extent by Na^+/Ca^{2+} exchanger (NCX) (Opperman et al. 1992; Bindels 1993; Friedman and Gesek 1995; Hong et al. 2004; Lee and Jeung 2007). Ca^{2+} serves as a universal intracellular messenger to modulate processes such as neurotransmission and hormone secretion as well as many biological processes, e.g., cell cycle regulation and programmed cell death (Berridge 1995; Clapham 1995).

In tissues, the plasma membrane Na^+/Ca^{2+} exchange proteins of mammals have been divided into two families, one in which Ca^{2+} action is dependent only on sodium (NCX family 1–3) and one in which Ca^{2+} action is also dependent on potassium (NCKX family 1–6) (Tsoi et al. 1998; Kraev et al. 2001; Li et al. 2002; Lytton et al. 2002; Dong et al. 2006).

Among the different pathways that mediate Ca^{2+} movement, Na^+/Ca^{2+} exchange has emerged as the predominant mechanism for Ca^{2+} efflux across the plasma membrane, particularly when overall Ca^{2+} levels are high (Lee et al. 2002; Wanaverbecq et al. 2003; Kim et al. 2005). Moreover, several recent studies have highlighted the connection between important physiological events and specific Na^+/Ca^{2+} exchange molecules (Jeon et al. 2003; Li et al. 2006; Pott et al. 2007). Extensive studies have demonstrated that Na^+/Ca^{2+} exchange plays a crucial role in Ca^{2+} extrusion and operates with a stoichiometry of three Na^+ ions to one Ca^{2+} ion, indicating that the transport process is electrogenic (Blaustein and Lederer 1999). The development of partially purified exchange preparations, antibody reagents, and expression cloning techniques led to the molecular cloning of the canine cardiac Na^+/Ca^{2+} exchanger, subsequently denoted NCX1 (Blaustein and Lederer 1999). NCX1 cDNA encodes a protein of 970 amino acids predicted to be approximately 110 kDa in size (Lytton 2007). The mature protein is subject to both signal peptide cleavage and glycosylation and, when analyzed on gels, runs with an apparent size of 120 kDa (Lytton 2007). Additional bands observed at 160 and 70 kDa arise by heat-induced aggregation and proteolytic cleavage, respectively. Further, minor variations in apparent size (5–10 kDa) are generated by alternative splicing (Lytton 2007). Parallel studies in retinal rod photoreceptors have established the presence of a mechanistically similar Na^+/Ca^{2+} exchange

process that is critical for visual adaptation and represent the predominant means of Ca^{2+} extrusion from rod outer segments (Schnetkamp 1986). The rod exchanger differs from that described in the heart and axon principally due to its absolute transport requirement for K^+ (Lytton 2007). Studies showed that the rod exchanger catalyzed the transport of four Na^+ in exchange for one Ca^{2+} and one K^+ (Cervetto et al. 1989; Schnetkamp et al. 1989). Purification of the bovine rod $Na^+/Ca^{2+}/K^+$ exchanger subsequently led to its molecular cloning (Cook and Kaupp 1988; Reilander et al. 1992). This cDNA, denoted NCKX1, encodes a protein of 1,216 amino acids predicted to be approximately 132 kDa in size (Lytton 2007). Extensive glycosylation of the protein appears to account for the much larger apparent size of the native protein observed on gels (≤220 kDa) (Lytton 2007). Both forward- and reverse-mode exchange occur via NCX and NCKX exchangers depending on the gradients of the Na^+/Ca^{2+} (and K^+) (Blaustein and Lederer 1999; Lytton et al. 2002). NCX1 is abundantly expressed in the heart, brain, kidney, and smooth muscle (Nicoll et al. 1990, 1996). However, NCX2 and NCX3 expression in the brain and skeletal muscle is limited (Longoni and Carafoli 1987; Nicoll et al. 1996; Kraev et al. 2001; Li et al. 2002; Lytton et al. 2002; Dong et al. 2006). NCKX1 is expressed only in retinal rod photoreceptors. NCKX2 in brain neurons and cone photoreceptors shows restricted expression (Kraev et al. 2001; Li et al. 2002; Lytton et al. 2002; Dong et al. 2006). NCKX3 and NCKX4 are expressed not only in the brain but also in many other tissues, including aorta, uterus, and intestine, which are rich in smooth muscle cells (Kraev et al. 2001; Li et al. 2002; Cai and Lytton 2004; Yang et al. 2009). NCKX5 has recently been demonstrated to be expressed in skin and retinal pigmented epithelium, where it is thought to be present on the melanosome membrane and not the plasma membrane (Lamason et al. 2005). NCKX6 has also been characterized; however, the physiologic function of this protein remains controversial (Cai and Lytton 2004; Palty et al. 2004). Studies examining the physiologic role(s) in vascular contraction via controlling Ca^{2+} homeostasis by NCKX and NCX proteins have been performed in blood vessels, and some recent reports describe NCKX and NCX function in brain, spermatozoa, mast cells, and platelets (Kiedrowski et al.. 2002; Aneiros et al. 2005; Kim et al. 2005; Kip et al. 2006). NCX1 and NCKX3 are expressed in the reproductive organs, including the uterus (Yang et al. 2009, 2011; Quednau et al. 1997; Kraev et al. 2001; Li et al. 2002). As mentioned previously, maintenance of the calcium balance of reproductive organs is of crucial importance for many physiologic functions, including smooth muscle contraction, embryo implantation, and placental transport. The regulation of contraction, muscle excitability, and the maternofetal calcium transport system of the placenta in the reproductive organs is important for lessening maternal and fetal mortality caused by pre- and postmature birth or metroparalysis (Wray et al. 2003). In addition, calcium plays an important role in the endometrium and placenta. A number of calcium-related proteins expressed in the uterus mediate muscle functions (Sanborn 2000) and in the placenta (Lafond et al. 1991; Moreau et al. 2003a; Yang et al. 2011) ensure successful implantation.

In this chapter, we summarize current findings related to the molecular mechanisms involved in NCX1 and NCKX3 regulation in mammals and introduce research data from our recent studies and others.

10.2 Na^+/Ca^{2+} Exchangers in Reproductive Tissues

The kidney plays an important role in the maintenance of calcium balance in both the male and female body by regulating calcium reabsorption and excretion. Ca^{2+} are filtered daily by the glomeruli, and <2% are excreted in the urine (Hoenderop et al. 2002b). In contrast, the transcellular pathway allows the body to regulate Ca^{2+} reabsorption independent of Na^+ balance. This pathway is specifically controlled by the calcitropic hormones, including parathyroid hormone, calcitonin, and 1,25-dihydroxy vitamin D_3 ($1,25[OH]_2D_3$) (Bindels 1993; Friedman and

Gesek 1995; Hoenderop et al. 2000, 2002a). Due to this active process, an organism can respond to fluctuations in dietary Ca^{2+} and adapt to changes in demand during certain processes, i.e., growth, pregnancy, lactation, and aging (Hoenderop et al. 2002a). Disturbances in active Ca^{2+} reabsorption are most likely to be accompanied by significant alterations in overall Ca^{2+} homeostasis (14). At the cellular level, active calcium transport can be divided into three functional steps, Ca^{2+} influx into cells, transferring through the cytosol, and extrusion into the bloodstream. These steps are mediated by three types of proteins: (1) the calcium entry channel proteins of the outer membrane, (2) cytosolic buffering or transfer proteins, and (3) excretory pump proteins (Hoenderop et al. 2002b; Van Cromphaut et al. 2003; Diepens et al. 2004; Choi and Jeung 2008). Two highly selective calcium channels on the apical sides of cells, members of the TRP superfamily of ion channels (TRPV6 and TRPV5), are the main Ca^{2+} entry channels (Hoenderop et al. 2002b; Nijenhuis et al. 2003). Calbindin-D9k (CaBP-9k) and Calbindin-D28k (CaBP-28k) are $[Ca^{2+}]_i$-binding proteins that are thought to participate in shuttling Ca^{2+} from the apical to the basolateral membrane, where the Na^+/Ca^{2+} exchanger and plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) mediate Ca^{2+} extrusion (Christakos et al. 2007; Choi and Jeung 2008). There has been great progress in our understanding of the mechanism of active calcium transport and its role in calcium-related disorders, such as hypocalcemia, rickets, and osteomalacia, using vitamin D receptor-null mice and 1α-hydroxylase-deficient mice (Van Cromphaut et al. 2001; Zheng et al. 2004). The role of several calcium-processing proteins in the active calcium transport system, including TRPV5/6, CaBP-9k/28k, PMCA1b, and NCX1, has been verified recently using gene knockout studies.

Previously, we reported on the phenotype of CaBP-9k-null mice and the effect of compensatory gene induction of calcium-related genes in these mice (Lee et al. 2007). We also examined the differential levels of NCKX3 mRNA and protein in the kidneys of male and female mice (Lee et al. 2002). Renal NCKX3 expression was certainly increased in male mice kidneys compared to those of female mice (Lee et al. 2002). Interestingly, several renal calcium-processing genes were highly expressed in both wild-type and CaBP-9k-null female mice in the absence of any treatment. In addition, several calcium-processing genes were altered in the kidneys and duodenum of both male and female mice by the same hormones; however, we did observe differential expression of the calcium-processing gene between males and females (Lee et al. 2002). We hypothesized that NCKX3 plays a more important role in maintaining the calcium balance in the female reproductive system than in Ca^{2+} metabolism in males. We investigated the importance or involvement of NCKX3 in the tissues of female reproductive organs. In several current studies, we examined whether Na^+/Ca^{2+} exchangers are differentially expressed in the reproductive organs of females. We also examined whether the regulation of Na^+/Ca^{2+} exchangers in female reproductive organs correlated with development and identified a potential mechanism involved in the relationship of female-specific diseases caused by expression of these genes.

10.3 Uterine Expression and Regulation of Na^+/Ca^{2+} Exchangers

The primate endometrium undergoes certain hormone-dependent changes, during a particular time window within the preimplantation phase, which prepare it to receive the growing blastocyst (Schlafke and Enders 1975; Yoshinaga 1988; Carson et al. 2000). A complex interaction between effector molecules, including steroid hormones, growth factors, and cytokines, regulates the development of a "receptive" state in the uterine epithelium (Psychoyos 1976, 1986; Sharkey 1998; Carson et al. 2000). The action of Ca^{2+} in female reproductive organs has been widely studied for several decades. It is suggested that Ca^{2+} is involved in uterine smooth muscle contraction and fetal implantation (Salamonsen et al. 2001; Daston and Naciff 2005; Dong et al. 2006). Also, the balance of Ca^{2+} during uterine contraction/relaxation is

extremely important throughout pregnancy and during labor. However, the mechanism underlying the regulation of calcium levels within uterine tissue remains largely unknown. While a physiological role for the NCKX and NCX proteins in the regulation of Ca^{2+} homeostasis during vascular and cardiac myocyte contraction has not yet been definitively established, recent reports describe NCKX and NCX function in brain, spermatozoa, mast cells, and platelets (Kiedrowski 2004; Kiedrowski et al. 2004; Aneiros et al. 2005; Kim et al. 2005; Kip et al. 2006). NCKX3 is expressed in the reproductive organs, including the uterus (Kraev et al. 2001; Yang et al. 2009); however, the specific role of NCKX3 within the uterus has not been fully characterized. Maintenance of calcium balance within the uterus is critically important for many physiological functions, including smooth muscle contraction during embryo implantation (Salamonsen et al. 2001; Luu et al. 2004). Thus, it is likely that the NCKX and NCX proteins may be functionally important in female reproductive organs.

In rodents, the expression of NCKX3 mRNA and protein fluctuates in the uterus during the cycle and is regulated by sex steroids (Yang et al. 2009). In mice, the uterine expression of NCKX3 mRNA and protein was highest at estrus, when the levels of E2 (17β-estradiol) and P4 (progesterone) are relatively low (Yang et al. 2009). However, rat uterine NCKX3 mRNA and protein was highly expressed in proestrus (Yang et al. 2011). Also, the levels of NCKX3 mRNA and protein were regulated by distinct sex steroids between mice and rats. In mice, uterine NCKX3 mRNA and protein expression was downregulated by both E2 and P4 when subcutaneously treated with the sex steroids of immature mice (Yang et al. 2009), and pretreatment with estrogen receptor (ER) or progesterone receptor (PR) antagonists completely recovered E2- or P4-mediated decreases in NCKX3 mRNA expression (Yang et al. 2009). In rats, uterine NCKX3 expression was induced by E2 and reduced by P4, and pretreatment with ER or PR antagonists restored the changed NCKX3 mRNA and protein levels to that of the untreated group (Yang et al. 2010).

In humans, endometrial NCKX3 expression is altered during the menstrual cycle and in an ER-positive endometrial cancer cell line (Ishikawa cell) (Boggess et al. 2006; Yang et al. 2009). Expression levels of NCKX3 mRNA change during the menstrual cycle, but that of NCX1 do not (Yang et al. 2011). The expression levels of NCKX3 during the early- and mid-proliferative phases and the early- secretory phase increase during the menstrual cycle; furthermore, endometrial expression of NCKX3 is lower than that in the early- and mid-proliferative phases and the early-secretory phase (Yang et al. 2011). It is known that the expression patterns of non-calcium-related proteins increase in the human endometrium during the early-secretory phase (Surveyor et al.1998; Marions and Danielsson 1999). In osteoblasts, these genes (COX-1 and COX-2) are regulated by extracellular calcium $[Ca^{2+}]_e$ via the extracellular signal-regulated kinase (ERK) signaling pathway (Choudhary et al. 2004). In pigs, significantly increased concentrations of calcium are observed in the uterine lumen during the implantation period (Geisert et al. 1982). Therefore, we speculated that the level of $[Ca^{2+}]_e$ observed in the human endometrium would be higher (Yang et al. 2011). The transporter function of NCKX has been demonstrated in the outer segments of rod photoreceptors, where NCKX1 is the principal mediator of Ca^{2+} extrusion (Schnetkamp 1995). Although the tissue is different, we hypothesized that NCKX3 induces Ca^{2+} extrusion to extracellular regions, that this high $[Ca^{2+}]_e$ concentration induces the expression of COX-1 and 2, and that these events may be involved in implantation. Na^+/Ca^{2+} exchangers are involved in a multitude of key points for the regulation of uterine function. These points of regulation are of fundamental importance to the function of the uterus, as large changes in contractile behavior are required to satisfy the demands on the tissue at different gestational states. Changes may also be expected in the expression of other proteins associated with the role of the sarcoplasmic reticulum (SR), as well as Ca^{2+} efflux mechanisms. Therefore, Ca^{2+} entry, together with SR Ca^{2+} release and efflux, optimizes the Ca^{2+} transient profile to the function of

the uterus. These reports showed that expression levels of NCKX3 in human endometrial tissues vary throughout the menstrual cycle. This fluctuation appears to be regulated by sex steroids, indicating that NCKX3 may be a major regulator of uterine function through its effects on fetal implantation and calcium homeostasis.

10.4 Placental Expression and Regulation of Na^+/Ca^{2+} Exchangers

Placental development depends on the transport of oxygenated maternal blood, nutrients, and mineral ions to the fetus. Fetal bone mineralization requires calcium and phosphorus exchange between maternal and fetal blood, and mammalian fetal nutrition is supplied by placental transfer of maternal nutrients during gestation (Hill and Longo 1980). The syncytiotrophoblast (ST) layer of the human placenta transfers as much as 30 g of Ca^{2+} from the mother to the fetus (Belkacemi et al. 2005). Calcium is actively transported across the placenta at an increased rate in late gestation to meet the needs of the rapidly mineralizing skeleton and to maintain a $[Ca^{2+}]_e$ that is physiologically appropriate for fetal tissues and that is higher than the maternal calcium concentration (Kovacs and Kronenberg 1997).

In the human placenta, NCX may play a significant role in trans-syncytial transfer and in regulating intracellular calcium $[Ca^{2+}]_i$ important for a variety of physiological mechanisms (Kamath and Smith 1994). There has been a controversy concerning the precise placental trophoblast membrane location of NCX. A minimal role of NCX in the trans-placental movement of Ca^{2+} from mother to fetus was observed in perfusion studies of human placental lobules (Williams et al. 1991). Human placental NCX expression was not shown in the basal plasma membrane (BPM) of STs (Lafond et al. 1991). The presence of NCX expression was demonstrated in BPM but not in brush-border membrane (BBM) (Kamath and Smith 1994). Expression of the widely distributed isoform NCX1 at the molecular level in whole human placental tissue has been reported (Kofuji et al. 1992). Recently, the presence of both NCX1 and NCX2 isoforms has been demonstrated in BeWo cells and in trophoblasts from human term placenta (Moreau et al. 2003a, b). The NCX3 gene product distribution pattern is normally restricted to the brain and skeletal muscle (Nicoll et al. 1990); its unexpected presence in trophoblasts may point to a specific role of this isoform in placental physiology (Moreau et al. 2003a, b). Under basal conditions, NCX does not have a major role in Ca^{2+} efflux (Moreau et al. 2003a, b). In fact, a minimal role of NCX in the trans-placental movement of Ca^{2+} from the mother to the fetus has been observed with perfusion of placental lobules (Williams et al. 1991). Therefore, NCXs are more likely to be active in cells where $[Ca^{2+}]_i$ levels are exposed to large variations (Moreau et al. 2003a, b). During human pregnancy, the mRNA expressions of NCKX3 and NCX1 were examined in the placenta (Yang et al. 2011). The patterns of human placental NCX1 mRNA expression were altered during the second and third trimester and rapidly increased at 40 weeks (Yang et al. 2011). However, placental NCKX3 mRNA expression did not evidently fluctuate during the late-second and late-third trimesters (Yang et al. 2011). During preterm labor, placental NCKX3 expression was highly expressed in the maternal section of human placenta compared to the fetal and central section; however, human NCX1 expression was not changed among the three sections of placenta (Yang et al. 2011). During term labor, NCKX3 expression was higher in the maternal section of placenta than the fetal and central sections, and the pattern of NCX1 expression was highest in the fetal section of the placenta compared to the other two sections (Yang et al. 2011). Localization of NCKX3 and NCX1 occurred in the cytoplasm of villous ST and inside of fetal and maternal plate layers, but not in extravillous cytotrophoblast and villous blood vessels (Yang et al. 2011). The calcium homeostasis proteins, NCKX3 and NCX1, were highly expressed in the cytoplasm of both ST and giant cells of the maternal plate in the human placenta (Yang et al. 2011). On the basis of these experiments, altered expression of NCKX3 and NCX1 during preterm or

term labor may affect maternofetal calcium absorption via syncytia of floating chorionic villi for $[Ca^{2+}]_i$ homeostasis and successful delivery.

In mice, mRNA and protein expression of NCX1 was significantly increased in two sections (central and fetal) of placenta compared to the maternal section (Koo 2012). In CaBP-9k and CaBP-28k knockout (KO) mice, the mRNA and protein expression of NCX1 was higher in all KO mice than in WT (wild-type) mice in all three sections of placenta (Koo 2012). Also, placental NCX1 expression was elevated in maternal to fetal sections of placental in WT, CaBP-9k, and CaBP-28k KO mice (Koo 2012). Localization of NCX1 protein was detected throughout the placenta along with expression in the STs of the labyrinthine zone and in fetal vascular endothelial cells in mice (Koo 2012). The process involves transepithelial Ca^{2+}/Na^+ transport, which is mediated by the ST (Stulc et al. 1993). The involvement of NCX in Ca^{2+} extrusion in epithelial cells is less well established. It may be that NCX is more important under conditions where $[Ca^{2+}]_i$ levels show large fluctuations, unlike the basal conditions examined in the current study (Moreau et al. 2003a, b). These results suggest that the compensatory expression of NCX1 is increased by ablation of CaBP-9k or CaBP-28k and that expression of NCX1 influences CaBP-9k and CaBP-28k expression. The enhanced expression of NCX1 in CaBP-9k and 28k KO mice may help compensate for CaBP deficiencies in the placenta.

10.5 The Relationship of Na^+/Ca^{2+} Exchangers in Reproductive Tissue Diseases

Diseases during pregnancy such as preeclampsia are characterized by maternal syndromes such as gestational hypertension, proteinuria, and in 30% of cases fetal syndromes such as reduced amniotic fluid and abnormal oxygenation. Preeclamptic placental oxidative stress, resulting from deficient remodeling of maternal spiral arteries, is of importance in preeclampsia. It induces the placenta to release various factors, such as inflammatory cytokines, apoptotic wastes, and anti-angiogenic factors, which change the intracellular environment (Redman and Sargent 2009). These secreted soluble factors are then thought to alter the endothelial metabolic status, mitochondrial integrity, and vascular functions including $[Ca^{2+}]_i$ behavior (Seta et al. 2004; Robinson et al. 2008). Increases in $[Ca^{2+}]_i$ are implicated in cell injury and death induced by hypoxic stress (Seta et al. 2004). Calcium entry blockers have been reported to protect against cellular necrosis caused by experimental ischemia in the liver, kidney, and other tissues (Peck and Lefer 1981; Lee and Lum 1986). In human placenta, oxidative stress exposes the placenta to fluctuating oxygen concentrations during preeclampsia (Kingdom and Kaufmann 1999). Effective calcium homeostasis is certainly required in hypoxic cells to maintain healthy cell environments for calcium homeostatic genes, such as Na^+/Ca^{2+} exchangers.

The most effective treatment for preeclampsia is delivery itself; however, several randomized trials report the effective use of various methods to reduce the rate or severity of preeclampsia (Sibai et al. 2005), such as Ca^{2+} supplementation. Several alterations in maternal Ca^{2+} homeostasis were identified in preeclampsia, such as low urinary Ca^{2+} excretion and low circulating levels of 1,25-dihydroxy vitamin D3, parathyroid hormone-related peptide, and calcitonin gene-related peptide (Seely et al. 1992; Halhali et al. 2000). Epidemiologic data suggest an inverse correlation between dietary Ca^{2+} uptake and the incidence of hypertensive disorders during pregnancy in diverse populations (Hofmeyr et al. 2007). Even though the results of two large clinical trials demonstrated disparity in the benefits with respect to Ca^{2+} supplementation in prevention of preeclampsia (Belizan et al. 1991; Levine et al. 1997), Ca^{2+} supplementation did reduce the risk of preeclampsia in high-risk pregnancies as well as in women with low baseline dietary Ca^{2+} uptake (Askie et al. 2007). This is a crucial element for adequate fetal development and prenatal programming of future diseases. Approximately 80% of the total fetal Ca^{2+} is accumulated during the last trimester of pregnancy. This Ca^{2+} transfer allows for adequate fetal skeleton mineralization (Pitkin 1983) and various cellular functions.

There are two pathways for Ca^{2+} entry into the fetus. Paracellular diffusion, active in perfused placental cotyledons, enables Ca^{2+} to cross the placental barrier and represents 66% of the total maternofetal Ca^{2+} transfer (Stulc et al. 1994).

Placental calcium transporting systems involve transepithelial transport, which is mediated by ST which is a polynucleated structure (Malassine and Cronier 2002) formed during implantation and represents the most important maternofetal barrier (Rasmussen 1986). Villous trophoblasts are continuously incorporated by syncytial fusion into the ST. Ca^{2+} absorption occurring in the placenta during pregnancy from the maternal blood pool to chorionic fetal arteries is the result of transport by two mechanisms, paracellular and transcellular (Khanal and Nemere 2008). Occludin and junction adherence molecular A (JAM-A), constructing tight junction and regulating paracellular transport, are needed for Ca^{2+} absorption. Paracellular Ca^{2+} absorption is highest in the ileum, while dietary Ca^{2+} absorption through transcellular mechanisms occurs predominantly in the duodenum (Khanal and Nemere 2008). The Ca^{2+} transepithelial transfer through the ST is passive-active transport and requires various proteins (Belkacemi et al. 2002, 2005). Ca^{2+} signaling pathways in the placenta are still under investigation, and there is very little information concerning the expression of these proteins in preeclamptic placental tissues.

NCKX3 mRNA and protein expression was induced in preeclamptic placenta compared to normal placenta in the fetal and maternal sections during preterm labor (Yang et al. 2011). Placental NCX1 mRNA and protein expression was higher in preeclamptic placenta than in normal placenta (Yang et al. 2011). However, during term labor, placental NCKX3 and NCX1 mRNA and protein expression were downregulated in all sections of preeclamptic placenta compared to normal placenta (Yang et al. 2011). These results suggest that altered expression of NCKX3 and NCX1 during preterm or term labor may affect maternofetal calcium absorption via syncytia of floating chorionic villi for $[Ca^{2+}]_i$ homeostasis and successful delivery.

In preeclamptic STs, unbalanced calcium ion gradients may be protected by the influence of oxidative stress on other factors and calcium transport proteins for safe fetal bone mineralization during pregnancy. In a future study, we plan to investigate the regulators of Na^+/Ca^{2+} exchangers and other calcium transporters in the placenta, which are produced from hypoxia-inducible factor-1α (HIF-1α) and unknown factors in the maternofetal calcium transport pathway. By elucidating factors involved in placenta Ca^{2+} transport during pregnancy, we are able to determine one of the possible mechanisms responsible for fetal predisposition to adult diseases related to preeclampsia. Although this fundamental approach will not have an immediate impact in clinical practice, it will help to characterize the Ca^{2+} transfer process from mother to fetus in pregnancy and evaluate whether or not it will ultimately be possible to improve antenatal placental transfer using nutritional intervention with pharmacological or hormonal agents.

10.6 Regulation of Na^+/Ca^{2+} Exchangers in Hypoxic Placental Cell Lines

Oxidative stress, resulting from deficient remodeling of spiral arteries, is an important aspect of preeclampsia. It induces the placenta to release various factors, such as inflammatory cytokines, apoptotic wastes, and anti-angiogenic factors (Redman and Sargent 2009). These secreted soluble factors are then thought to alter endothelial metabolic status, mitochondrial integrity, and vascular functions (Robinson et al. 2008). An increase in $[Ca^{2+}]_i$ is implicated in cell injury and death induced by hypoxic stress (Seta et al. 2004). Calcium entry blockers have been reported to protect against cellular necrosis caused by experimental ischemia in liver, kidney, and other tissues (Peck and Lefer 1981; Lee and Lum 1986). In human placenta, oxidative stress exposes the placenta to fluctuating oxygen concentrations during preeclampsia (Kingdom and Kaufmann 1999).

In hypoxic placental cells, to assess whether Ca^{2+} transport and homeostasis is affected by hypoxia, we examined Na^+/Ca^{2+} exchangers NCKX3 and NCX1 in placental cell lines, i.e., BeWo, JEG3, and human placental primary cells (hPC). The hPC were obtained from normal placenta (7–12 week of gestation) (Yang et al. 2011). The expression levels of Na^+/Ca^{2+} exchanger mRNA and protein were increased in hPC by oxidative stress, and distinct expression patterns in BeWo and JEG3 cells were found during hypoxia (Yang et al. 2011). TRPV6 mRNA expression was not altered by oxidative stress in BeWo cells, but in JEG3 cells, the level of TRPV6 mRNA was decreased during hypoxia. In addition, PMCA1 mRNA expression was downregulated by oxidative stress in both BeWo and JEG3 cells. The expression of NCKX3 and NCX1 mRNA and protein were increased by oxidative stress in all placental cell lines.

In the ER-positive endometrial carcinoma Ishikawa cell line, NCKX3 mRNA and protein expression was elevated by E2, and induced NCKX3 expression was completely inhibited by the ER-specific antagonist, ICI 182780 (Yang et al. 2011). We also examined the effects of E2 on NCKX3 expression in an ER-negative endometrial carcinoma cell line (RL95). We did not observe a differential expression pattern of NCKX3 in E2-treated RL95 cells (not published data). These results suggest that distinct expression patterns of NCKX3 between ER-positive and ER-negative cell lines may be involved in ER-mediated NCKX3 transcripts in human endometrial ER-positive cells (Ishikawa).

In Chinese hamster ovary cells (CHO), Na^+-dependent inactivation of NCX is manifested in transfected CHO cells as a reduced V_{max} for Ca^{2+} uptake with no change in K_h for allosteric Ca^{2+} activation (Chernysh et al. 2008). The WT canine exchanger used in these studies was quite resistant to Na^+-dependent inactivation, even after extensive phosphatidylinositol-4,5-bisphosphate (PIP_2) depletion, but was strongly inactivated when pH_i was reduced. The resistance of the WT exchanger to Na^+-dependent inactivation suggests that this mode of NCX regulation is of little importance under normal physiologic conditions. Na^+-dependent inactivation could be an important protective response during ischemia, when high $[Na^+]_i$, low ATP/PIP_2, and low pH_i would strongly promote inactivation, thereby reducing NCX-mediated Ca^{2+} influx and toxic Ca^{2+} overload (Chernysh et al. 2008).

On the basis of these experiments, we confirmed that the expression level of Na^+/Ca^{2+} exchangers in human, bovine, and CHO cells varied throughout the reproductive cycle and Na^+-dependent inactivation. This universal expression of Na^+/Ca^{2+} exchangers appears to be regulated by sex steroid (E2) extracellular ion activation, indicating that NCKX3 and/or NCX1 may be a major regulator of uterine and placental functions by extracellular and intracellular Na^+, K^+ activation, and plasma membrane ER via effects on fetal implantation and calcium homeostasis.

10.7 Conclusion

Calcium, among all ions, is one of great importance since it is known to be implicated in many physiological processes. This chapter is an overview of most endometrial and placental proteins involved in calcium homeostatic exchangers during pregnancy. Many hormones and growth factors are involved in placental and fetal development and uterine endometrial recovery. The ST and endometrial epithelial cells, specialized cells in calcium metabolism of reproductive tissues involved in calcium regulation and transport, are also greatly influenced by these factors in the uteroplacental environment. However, the complex calcium movement in the ST necessitates many structures that have specific functional roles in this process. To date, much of our knowledge is fragmentary. In the placenta, the physiologic and clinical relevance of NCKX3 and NCX1 are still unclear. Studies at the whole animal level looking at regulatory proteins that interact with these Na^+/Ca^{2+} exchanger channels and control their functions will certainly contribute to our understanding of the pathophysiologic significance of the expression of these channels in the placenta and uterus. It is hypothesized that

active transcellular calcium transport proceeds through a well-controlled sequence of events consisting of apical calcium entry via TRPV5 and TRPV6, present only in BBM, its cytosolic diffusion through its binding to CaBPs, and its basolateral extrusion mainly through PMCA and to a lesser extent by NCX. Moreover, the involvement of VDCCs, store-operated calcium entry (SOC) in Ca^{2+} regulation of hormonal secretions in placental trophoblasts and endometrial glands, and epithelial cells certainly represents interesting possibilities and warrants further investigation. Consequently, it is reasonable to speculate that useful areas of future investigations in the placenta and uterus are likely to include (1) identification of the regulatory domains in these exchangers with the signaling pathways controlling their activity and (2) the use of inhibitors of some channels for pharmacological manipulation in several disorders related to Ca^{2+} homeostasis.

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