Ca\(^{2+}\)-ATPase protein expression in mammary tissue

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Reinhardt, Timothy A., Adelaida G. Filoteo, John T. Penniston, and Ronald L. Horst. Ca\(^{2+}\)-ATPase protein expression in mammary tissue. Am J Physiol Cell Physiol 279: C1595–C1602, 2000.—Protein expression of plasma membrane Ca\(^{2+}\)-ATPases (PMCA) and the putative Golgi secretory pathway Ca\(^{2+}\)-ATPase (SPCA) was examined in rat mammary tissue. As lactation started, PMCA protein expression increased dramatically, and this increased expression paralleled milk production. Mammary PMCA was primarily PMCA2b but was ~4,000 daltons larger than expected. RT-PCR showed that the primary mammary PMCA2b transcript was alternatively spliced, at splice site A, to include an additional 135 bp, resulting in the insertion of 45 amino acids. This splice form is designated 2bw. PMCA2bw is secreted into milk, associated with the milk fat globule membrane. Therefore, PMCA2bw is located on the apical membrane of the secretory cell. Smaller amounts of PMCA1b and 4b protein were found in mammary tissue. PMCA4b was the major PMCA expressed in developing tissue, and its level declined as lactation started. SPCA protein expression increased moderately during lactation. SPCA protein expression increased 1 wk before parturition and increased further as lactation proceeded. The abundance and cell location of PMCA2b suggest that it is important for macro-Ca\(^{2+}\) homeostasis in lactating tissue. The pattern of expression and abundance of SPCA suggest that it is a candidate for the Golgi Ca\(^{2+}\)-ATPase.

Ca\(^{2+}\)-ATPase protein expression in mammary tissue is thought to be important for normal protein synthesis, processing, and secretion (8, 15). Therefore, a mechanism must be in place to move Ca\(^{2+}\) into the Golgi.

Despite the importance of mammary cell Ca\(^{2+}\) homeostasis, little is known about the proteins involved in Ca\(^{2+}\) transport and their regulation (44). Biochemical evidence exists for a P-type Ca\(^{2+}\)-ATPase in the Golgi of mammary tissue with characteristics slightly different from either the plasma membrane Ca\(^{2+}\)-ATPase (PMCA) or the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) (4, 5, 15, 40, 50). This mammary Golgi secretory pathway Ca\(^{2+}\)-ATPase (SPCA) could be a homologue to the yeast Golgi Ca\(^{2+}\)-ATPase PMR1 (16, 45), such as the putative rat SPCA (26). It has also been suggested that there is no unique mammalian Golgi Ca\(^{2+}\)-ATPase but, rather, that SERCAs in the endoplasmic reticulum (ER) and PMCA in transit to the plasma membrane maintain the Ca\(^{2+}\) concentrations found in the Golgi (49). Previous work has shown that the putative rat SPCA mRNA is expressed in the mammary gland and is associated with milk production (44). Furthermore, the pattern of SPCA expression is consistent with the biochemical evidence for a Golgi SPCA in lactating tissue.

In addition to being pumped into the Golgi, Ca\(^{2+}\) must be effectively removed from the mammary cell and pumped into the milk. PMCA2b represent a ubiquitous, high-affinity system for removal of Ca\(^{2+}\) from the cell. PMCAs, along with SERCAs, are responsible for the long-term setting and maintenance of intracellular Ca\(^{2+}\) levels (1, 11–13, 23, 28). Mammalian PMCAs are encoded by a multigene family consisting of four members termed PMCA1-4 (7, 11, 23). Cloning has revealed some 20–30 isoforms of the pump, generated by alternative splicing of the primary gene transcripts at two sites identified as A and C (2, 20, 27, 28, 34, 38, 48). Alternative splicing of site C (COOH-terminal tail) has been shown to alter the regulatory properties of PMCA isoforms, particularly with respect to phosphorylation and calmodulin stimulation (17, 18, 23). Alternate splicing at site A has been shown to occur in PMCA2 (2, 27). No specific function has yet been found for this splice site (28), and only the z splice form of the protein has been demonstrated (20). The distribution of PMCA mRNA in rat and human tissues reveals that gene products 1 and 4 are ubiquitous, whereas gene

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products 2 and 3 are tissue specific and are found enriched in nervous tissue (22). Early protein studies have confirmed this pattern of expression for the four isoforms. It has also been shown that the splice variants of each isoform are expressed in a tissue- and cell type-specific manner (20, 23, 46–48).

Previous work has shown that PMCA isoforms 1b, 2b, and 4b, but not 3, are expressed in rat mammary tissue (44). PMCA2b mRNA was the most abundant PMCA mRNA expressed, and its expression was high in the lactating mammary gland of the rat (44). These data suggested that PMCA2b mRNA expression in the mammary gland is highly regulated by a factor(s) controlling milk production. This is the first evidence of significant expression of the PMCA2 isoform in a tissue other than brain. Thus it is suggested that PMCA2b might play a key role by regulating intracellular Ca\(^{2+}\) fluxes where large transcellular Ca\(^{2+}\) fluxes are required for milk production.

In this paper we present evidence for high expression of SPCA and PMCA2b protein in rat lactating mammary tissue. Interestingly, we have evidence that alternate splicing at site A in the mammary gland is different than that observed in the brain, where the shorter z splice form of PMCA2b is predominant (2, 20). We will also demonstrate that the PMCA2bw transcript and the correspondingly larger 2bw protein are the primary PMCA forms observed in lactating mammary tissue, whereas PMCA4b is the primary PMCA protein in developing mammary tissue.

**MATERIALS AND METHODS**

**Animals.** The National Animal Disease Center’s Animal Care and Use Committee approved all animal procedures. Confirmed-pregnant Sprague-Dawley rats were purchased from Harlan Sprague Dawley (Madison, WI). Rats were housed, individually, in hanging basket cages with sawdust bedding. Starting 11 days after the breeding date, rats were killed at 10, 7, and 1 day prepartum and 1, 7, 14, 18, and 21 days postpartum. Data are presented as days, with parturition as the reference point: negative days indicate days before parturition, and positive days indicate days of lactation (i.e., after parturition). Rats were anesthetized with a 50:50 mix of pentobarbital and a-xylene and centrifuged at 4,000 g.

**Buffer C**

discarded, and the pellets were resuspended in 2 (containing 0.15 M KCl). Membrane preparations were centrifuged at 100,000 g and adjusted to 0.7 M KCl by the addition of solid KCl, an equal volume of buffer A, containing 0.3 M KCl and centrifuged at 10,000 g for 15 min. This step was repeated one to two times or until the supernatant was clear. The washed pellet was processed in the same manner as tissue from this point forward to yield milk fat globule membranes (MFGMs). Proteins were determined with the Bio-Rad Protein Assay Kit using a BSA standard.

**Gel electrophoresis and Western blotting.** The methods were basically as described previously (20). Briefly, microsomes were incubated for 15 min at room temperature in a modified Laemmli buffer containing 150 mg/ml urea and 65 mM dithiothreitol. Samples were then electrophoresed for 1 h at 125 V in a 6% Tris-glycine gel (Novex, San Diego, CA). Proteins were transferred to nitrocellulose membranes for 1 h at 25 V in 0.7 M glycine-0.025 M Tris at pH 7.4. Blots were developed with Supersignal (Pierce, Rockford, IL) by using the protocol provided by the manufacturer. Anti-PMCA antibodies 5F10, NR1, NR2, and NR4, described previously (20), were used at 1:20,000, 1:1,000, 1:1,000, and 1:5,000 dilutions, respectively. Antibody 220 (final dilution 1:5,000) is a PMCA2-specific antibody similar to the previously described NR2 (20). Antibody 220 was prepared by using the same peptide sequence (TNSDFYSKNNQRNESSC) used to make NR2. The peptide sequence used to make the SPCA antibody (final dilution 1:5,000) was VARFQKIPNVENETMIC (26). Rabbits were immunized with peptide, which was synthesized by Multiple Peptide Systems (San Diego, CA) and conjugated to keyhole limpet hemocyanin. The general procedures were described previously (35). Antibodies 220 (anti-PMCA2) and anti-SPCA were characterized as follows. Antibody 220 gave the same results as the previously characterized NR2, and preincubation of antibody 220 with the PMCA2 peptide TNSDFYSKNNQRNESSC eliminated specific banding on Western blots. Preincubation of antibody 220 with an irrelevant peptide yielded normal PMCA2 blots. Preincubation of SPCA antibody with the SPCA peptide VARFQKIPNVENETMIC eliminated specific banding on Western blots. Preincubation of the SPCA antibody with an irrelevant peptide yielded normal SPCA blots with bands of the predicted size.

**RNA preparation and Northern blot analysis.** Total RNA and DNA were isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method using Trizol reagent (GIBCO-BRL, Gaithersburg, MD). Poly(A)\(^{+}\) RNA was prepared using the Poly A Tract mRNA Isolation System IV (Promega, Madison, WI). Poly(A)\(^{+}\) RNA (2–3 μg) was fractionated in formaldehyde-agarose gels, and blotting and hybridization were performed as previously described (31). PMCA2 and SPCA probes were prepared by RT-PCR as described previously. After hybridization, the blots were imaged and quantitated on an InstantImager (Packard Instruments, Downers Grove, IL).

**RT-PCR and sequencing.** The primers used for RT-PCR of splice A in PMCA2b were 5’-TGAAAGCTGCTCA-CAGGG-3’ and 5’-TCAGAGGCTGCATTTCCATAG-3’, as described previously (2). PCR was carried out in a GeneAmp 9600 PCR System (Perkin Elmer, Foster City, CA). The RT-PCR reaction master mix (99 μl) consisted of 10 μl of 10× reaction buffer (Boehringer Mannheim), 77.75 μl of diethyl pyrocarbonate-treated water, 8 μl of dNTPs of 10 mM stock (Amresco, Solon, OH), 1 μl at 100 pmol/μl of each of the specific primers (IDT, Coralville, IA), 0.25 μl (50 units) MMLV (GIBCO-BRL), 0.5 μl (20 units) RNAsin (Promega), and 0.5 μl (2.5 units) Taq polymerase (GIBCO-BRL). To this master mix we added 1 μl of template (1 μg RNA). The RT

**DNA isolation.** Total DNA was isolated from liver, brain, mammary gland, and placenta using a modified DNeasy kit (Qiagen, Valencia, CA). The DNA was resuspended in 10 mM Tris, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 4 μg/ml aprotinin, and 4 μg/ml leupeptin at pH 7.5. An aliquot of the homogenate was mixed with 50 μl of PBS plus complete protease inhibitor cocktail (Boehringer Mannheim, Indianapolis, IN), and centrifuged at 70°C until assayed.
reaction was incubated for 40 min at 56°C, followed by 4 min at 95°C. PCR was 25 cycles at 94°C for 10 s, 60°C for 30 s, and 72°C for 30 s. The run was completed with a 20-min extension step at 72°C. PCR products of the expected band size were sampled and reamplified using the same primers. The reamplified products were purified and submitted to the Iowa State University Sequencing Facility for direct PCR sequencing.

Statistical analysis. Differences in pump expression, as affected by day, were analyzed by least-squares ANOVA. When day effects were significant, specific comparisons were made using Tukey’s test. All analysis was performed using StatView (SAS Institute, Cary, NC).

RESULTS

PMCA expression in rat mammary tissue as affected by pregnancy and lactation. The patterns of Ca\(^{2+}\)-ATPase protein expression in mammary tissue of rats 11 days pregnant to 21 days lactating were examined by Western blot. Figure 1A shows a typical blot representing the expression of total PMCA. Using an antibody (5F10) that recognizes all PMCA isoforms, we found small amounts of PMCA protein in the mammary gland before parturition. It was not until lactation started that total PMCA protein increased significantly \((P < 0.1)\). PMCA protein expression increased 15 times by day 18 of lactation \((P < 0.001)\) compared with that at day 11 of pregnancy (Fig. 1). This increase in total PMCA paralleled milk production. Using isoform-specific antibodies (Fig. 2), we found that PMCA1b protein was present at low levels on day 10. PMCA1b protein doubled in late pregnancy and almost doubled again by peak lactation on day 18 \((P < 0.01)\) (Fig. 2A). As shown in Fig. 1, the developing mammary gland of pregnant rats expressed relatively low amounts of PMCA compared with the levels expressed in the lactating mammary gland. Figure 2B also shows that this relatively small amount of PMCA in developing mammary tissue is primarily PMCA4b. PMCA4b expression increased 2.5 times \((P < 0.05)\) from day 11 of pregnancy to day 1 of lactation. As lactation increased, PMCA4b expression in the mammary gland declined significantly \((P < 0.001)\). Since PMCA1b (Fig. 2A) increased only moderately during lactation, while PMCA4b was rapidly declining in lactation (Fig. 2C), the large increase in PMCA cannot be attributed to isoforms 1 and 4. Probing the blots with anti-PMCA2 antibodies showed that the large increase in PMCA protein expression is due primarily to PMCA2b (Fig. 2B). PMCA2b protein expression increased significantly on day 1 of lactation \((P < 0.001)\) and increased almost 100 times by day 18 of lactation \((P < 0.001)\) compared with that at day 11 of pregnancy.

**Fig. 1.** A: general plasma membrane Ca\(^{2+}\)-ATPase (PMCA) protein expression in rat mammary tissue during pregnancy and lactation, as determined by Western blot with the use of an antibody (5F10) that recognizes all PMCA isoforms. B: normalized expression pattern changes expressed as a percentage of expression relative to that on day \(-10\) \((\text{per } \mu\text{g tissue DNA}).\) Data are means ± SE \((n = 3–5\) animals tested on 3–5 gels) presented in days, with parturition as the reference point: negative days indicate days before parturition, and positive days indicate days of lactation (i.e., after parturition).

**Fig. 2.** Top: PMCA1, 2, and 4 protein expression in rat mammary tissue during pregnancy and lactation, as determined by Western blot using antibody NR1, which recognizes PMCA1 (A), antibody 220, which recognizes PMCA2 (B), and antibody JA9, which recognizes PMCA4 (C). Bottom: normalized expression pattern changes expressed as a percentage of expression relative to that on day \(-10\). Data are means ± SE \((n = 3–5\) animals tested on 3–5 gels) presented in days.
Evidence for alternate splicing of PMCA2b, at splice site A, in mammary tissue. Western blotting showed that mammary PMCA2b was ~4,000 daltons larger than expected compared with brain PMCA2b (Fig. 3A) or recombinant PMCA2bz (data not shown). RT-PCR showed that the primary mammary PMCA2b transcript was alternatively spliced, at splice site A, to include an additional 135 bp resulting in the insertion of 45 amino acids (Fig. 3B). This splice form is designated 2bw and accounts for the 4,000-dalton increase observed for mammary PMCA2b. The identity of the PCR products in Fig. 3 was confirmed by size and sequencing. RT-PCR showed that the w splice form was the predominant PMCA2 transcript in the mammary gland, kidney, uterus, and liver (Fig. 3B). However, only the mammary gland expressed PMCA2bw protein in abundance, with small amounts of PMCA2bw seen in the liver (Fig. 3A).

Figure 4 shows a comparison of transcript and protein expression of PMCA2 in mammary gland and brain. Mammary gland expresses four times more PMCA2b mRNA per microgram of DNA than the brain, but the brain expresses three to four times more PMCA2b protein. The other tissues tested expressed very small amounts of PMCA2b transcript and protein per microgram of DNA. The finding of greater PMCA2b mRNA in the mammary gland, but less protein than in the brain, suggests a significantly shorter half-life for PMCA2b in the mammary gland compared with the brain.

Putative (Golgi) SPCA expression in rat mammary tissue as affected by pregnancy and lactation. Using an antibody specific for the putative rat SPCA, we found that SPCA protein expression was evident in the mammary gland at day 11 (day ~10) of pregnancy (Fig. 5). SPCA protein expression increased three times (P < 0.01) by day 14 (day ~7) of pregnancy. SPCA protein increased further at the start of lactation and remained elevated (P < 0.001) at six to seven times the level seen at day 11 of pregnancy through day 18 of lactation (Fig. 5, A and B). Western blotting showed that a form of mammary SPCA was ~2,000 daltons larger than expected when compared with brain or testes SPCA (Fig. 5C). RT-PCR, with primers covering most of the open reading frame (bases 205–2,858) of rat SPCA (GenBank accession no. M93017), failed to find evidence for an alternatively spliced form of SPCA. This suggests that alternate splicing occurs outside the region examined or that the increased size of mammary SPCA is due to posttranslational modifications. Preincubation of the SPCA antibody with the SPCA peptide used to prepare the antibody eliminated specific banding on the Western blots (data not shown). The data suggest a mammary-specific SPCA splice form because the mammary form was not found in any other tissues tested. The splice must occur at the extreme amino or carboxyl terminus.

Figure 6 shows that the mammary gland expressed seven to eight times more SPCA mRNA per microgram of DNA than did the brain but only two to three times more SPCA protein than the brain. While this difference is not as dramatic as that seen for PMCA2 (Fig. 4), it does suggest that SPCA protein is turning over slightly faster in the mammary gland than in the brain.

Secretion of Ca$^{2+}$-ATPases into milk on the MFGMs. The fat in milk is a lipid droplet surrounded by a membrane. The source of this membrane is the apical membrane of the milk secretory cell. Therefore, examination of MFGMs yields information about the proteins located on the apical membrane of the mammary secretory cell. Using antibody 5F10, which recognizes
all PMCA isoforms, we showed that significant amounts of PMCA were associated and secreted into milk with the MFGM (Fig. 7, top left). PMCAs in MFGMs were enriched eight times (Fig. 7, bottom) more than PMCA measured in mammary tissue membranes (see Fig. 7 legend for protein loading differences between blots and membrane sources). Anti-PMCA2 antibody showed that PMCA2 was 21 times more concentrated in MFGMs than in mammary tissue membranes. Small amounts of PMCA1b and 4b were associated with the MFGM (data not shown), but PMCA2 accounted for the majority of PMCA in MFGMs. In contrast to the enrichment of PMCAs in MFGMs, SPCA (Fig. 7, top right) was found to be primarily associated with mammary membranes with very little SPCA found associated with MFGMs.

Comparison of Ca\(^{2+}\)-ATPases expression in mammary tissue to other tissues. Figure 8 shows the relative expression of PMCAs, PMCA2, PMCA4, and SPCA in mammary tissue compared with that in the brain, kidney, uterus, liver, and intestine (note different amounts on membranes loaded by tissue type). Except for the brain, the lactating mammary gland expresses more Ca\(^{2+}\)-ATPases than most tissues.

**DISCUSSION**

The lactating mammary gland is the site of significant Ca\(^{2+}\) storage and transport, but despite the importance of mammary cell Ca\(^{2+}\) homeostasis, little is known about the proteins that regulate it or their expression (29, 41). Previously, we identified the Ca\(^{2+}\)-ATPases (PMCA1b, 2b, 4b, and SPCA) present in mammary tissue and examined their mRNA expres-

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**Fig. 5.** A: secretory pathway Ca\(^{2+}\)-ATPase (SPCA) protein expression in rat mammary tissue during pregnancy and lactation, as determined by Western blotting. B: changes in SPCA expression relative to that on day -10. Data are means ± SE (n = 3–5 animals tested on 3–5 gels) presented in days. C: a Western blot of brain, mammary gland, and testes performed with an SPCA-specific antibody. The amount of membrane protein loaded was 8 µg of brain, 15 µg of mammary tissue, and 25 µg of testes membrane protein.

**Fig. 6.** Relative expression of SPCA mRNA (top) and protein (bottom) in brain, lactating mammary tissue, and testes.

**Fig. 7.** Western blots comparing mammary gland microsomes (lane 1) and milk fat globule membranes (lane 2) isolated from rats on day 14 of lactation. For the blots performed with the general PMCA antibody (5F10) and the anti-SPCA antibody, lane 1 was loaded with 15 µg of mammary membrane protein and lane 2 was loaded with 25 µg of milk fat globule membrane protein. The anti-PMCA2 blot had 30 µg of mammary membrane protein in lane 1 and 5 µg of milk fat globule membranes in lane 2. The table shows the relative abundance of pump types in each membrane preparation and the enrichment, if any, in the milk fat globule membranes.
protein was PMCA2, similar to findings in cultured granule cells at peak development.

The significant expression of PMCA2 transcripts and protein in lactating mammary tissue (Figs. 2 and 3) is contrary to the popular belief that PMCA2 is only present in significant amounts in brain (23). Figure 4 shows that PMCA2 mRNA in the mammary gland is four times that observed in brain, whereas PMCA2 protein levels are one-third that seen in brain. The higher mRNA but lower PMCA2 protein seen in mammary tissue vs. brain suggests a much shorter half-life for mammary PMCA2 than the reported 12-day half-life for brain PMCA2s (19). A significantly shorter half-life for PMCA2 protein in the mammary gland is supported by the finding that PMCA2 is concentrated and secreted into the milk on the MFGM (Fig. 7). The source of MFGM is the apical membrane of the mammary secretory cell. This apical membrane surrounds milk fat as it is secreted into milk, and it is estimated that the apical membrane is completely lost and replaced three times a day as a part of the process of milk fat secretion (21, 32, 33). Consequently, the high expression of PMCA2 mRNA in the mammary gland is explained by the need to rapidly replace PMCA2 lost to milk secretion. The concentration of PMCA2 in the MFGM suggests that PMCA2 is primarily located on the apical membrane. The mammary secretory cell transports Ca\(^{2+}\) from the basolateral to the apical side of the cell. The apical location of most of the mammary PMCA contrasts with that in the kidney and intestine, where PMCA2s are localized in the basolateral membrane and are also involved in macrotomcellular Ca\(^{2+}\) fluxes (6, 51). This raises the question as to how PMCA2s might be selectively targeted to apical or basolateral membranes in polarized epithelial cells.

Isoform-specific antibodies showed that mammary PMCA2 was primarily PMCA2b but that it was ~4,000 daltons larger than expected. RT-PCR showed that the primary mammary PMCA2b transcript was alternatively spliced, at splice site A, to include an additional 135 bp resulting in the insertion of 45 amino acids. This splice form is designated 2bw (2) and accounts for the 4,000-dalton increase in size of mammary PMCA2b. Only the mammary gland expressed PMCA2bw protein in abundance, with small amounts of PMCA2bw found in the liver (Fig. 3). The significance of this splice form remains to be determined because no functional differences resulting from alternate splicing at site A have been detected with the methods used (28). Although its role is yet unknown, the w splice form could play a role in targeting PMCA2 to the apical membrane of the mammary secretory cell. The putative Golgi Ca\(^{2+}\)-ATPase, SPCA, is the only protein that increased significantly before parturition (Fig. 5). This finding is similar to results in our previous study in which the mRNA for SPCA was the only transcript to increase before parturition (44). This is significant because this is the time when the animal becomes hypocalcemic due to rapid influx of Ca\(^{2+}\) to mammary stores. Clear biochemical evidence exists for a Golgi Ca\(^{2+}\)-ATPase important to mammary function.
in general and casein micelles synthesis in particular (4, 5, 8, 15, 39, 40, 50). In yeast, the PMR1 gene product is a Ca\(^{2+}\)-ATPase located in the Golgi complex of yeast (3, 16, 42, 45). Rat, human, and bovine homologues of the yeast PMR1 have been detected by cloning, but their subcellular location and specific function remain to be determined (26, 30). Mutations in the human PMR1 homologue have been shown to be the cause of Hailey-Hailey disease, in which cultured keratinocytes from these patients have impaired regulation of cytoplasmic Ca\(^{2+}\) (30). Together, the data for yeast and humans suggest that the mammalian PMR1 homologue could be a mammalian Golgi Ca\(^{2+}\)-ATPase. The opposing argument put forth by Taylor et al. (49) is that the Golgi complex does not contain a unique “resident” Ca\(^{2+}\)-ATPase. They state that all Ca\(^{2+}\) uptake into Golgi can be attributed to PMCA2s in transit to the plasma membrane and to SERCs that are not restricted to the ER. The presence of a unique resident Ca\(^{2+}\)-ATPase in the Golgi remains to be determined, but data presented here and elsewhere suggest that SPCA is a candidate (26, 30, 44, 45).

Whether SPCA is determined to be a Golgi Ca\(^{2+}\)-ATPase or not, transport and retention of calcium by the Golgi is important to mammary cell function (8, 15) and to cell function in general (36, 37, 43). As noted above, the high expression of PMCA2 mRNA in the mammary gland is explained by the need to rapidly replace PMCA2 protein lost to milk secretion. This need to rapidly replace PMCA2 lost to the rapid turnover of the apical membrane of the mammary secretory cell also should result in significant PMCA2 in transit in the Golgi. It is reasonable, on the basis of the findings of Taylor et al. (49), that the large amount of PMCA2 in transit to the apical membrane may make a significant contribution to Golgi Ca\(^{2+}\) in the mammary gland. The contribution of PMCA2s to Golgi Ca\(^{2+}\) would appear to be restricted to after the start of lactation, because there is little PMCA2 present before significant milk production (Fig. 2). The mammary gland needs to accumulate Ca\(^{2+}\) before the initiation of lactation. The significant expression of SPCA before the start of parturition and lactation is therefore significant, because this putative Ca\(^{2+}\)-ATPase is the only Ca\(^{2+}\)-ATPase to increase significantly before parturition.

In summary, this study demonstrates that the initiation of lactation and increasing milk production are associated with significant PMCA expression in the mammary gland. The primary PMCA expressed is the unique isoform PMCA2bw. PMCA2bw is apparently synthesized at a high rate to maintain its concentration in the apical membrane of the secretory cell, where it is rapidly lost to milk secretion. The abundance, cell location, high affinity for Ca\(^{2+}\), and high constitutive activity of PMCA2bw suggest that PMCA2b is important for macro-Ca\(^{2+}\) homeostasis in lactating tissue. The putative Golgi Ca\(^{2+}\)-ATPase, SPCA, is the only Ca\(^{2+}\)-ATPase whose expression increases significantly before parturition at a time of significant Ca\(^{2+}\) accumulation by the mammary gland. The pattern of expression and the abundance of SPCA suggest that it is a candidate for the Golgi Ca\(^{2+}\)-ATPase shown to be important in maintaining the Golgi Ca\(^{2+}\) concentration required for casein synthesis. Several questions remain to be addressed, specifically with regard to the nature and function of SPCA and whether the large amount of PMCA2bw being made contributes to Golgi Ca\(^{2+}\) as it transits to the plasma membrane.

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