TRIENNIAL LACTATION SYMPOSIUM/BOLFA: Serotonin and the regulation of calcium transport in dairy cows¹

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ABSTRACT: The mammary gland regulates maternal metabolism during lactation. Numerous factors within the tissue send signals to shift nutrients to the mammary gland for milk synthesis. Serotonin is a monoamine that has been well documented to regulate several aspects of lactation among species. Maintenance of maternal calcium homeostasis during lactation is a highly evolved process that is elegantly regulated by the interaction of the mammary gland with the bone, gut, and kidney tissues. It is well documented that dietary calcium is insufficient to maintain maternal calcium concentrations during lactation, and mammals must rely on bone resorption to maintain normocalcemia. Our recent work focused on the ability of the mammary gland to function as an accessory parathyroid gland during lactation. It was demonstrated that serotonin acts to stimulate parathyroid hormone-related protein (PTHrP) in the mammary gland during lactation. The main role of mammary-derived PTHrP during mammalian lactation is to stimulate bone resorption to maintain maternal calcium homeostasis during lactation. In addition to regulating PTHrP, it was shown

that serotonin appears to directly affect calcium transporters and pumps in the mammary gland. Our current working hypothesis regarding the control of calcium during lactation is as follows: serotonin directly stimulates PTHrP production in the mammary gland through interaction with the sonic hedgehog signaling pathway. Simultaneously, serotonin directly increases calcium movement into the mammary gland and, subsequently, milk. These 2 direct actions of serotonin combine to induce a transient maternal hypocalcemia required to further stimulate PTHrP production and calcium mobilization from bone. Through these 2 routes, serotonin is able to improve maternal calcium concentrations. Furthermore, we have shown that Holstein and Jersey cows appear to regulate calcium in different manners and also respond differently to serotonergic stimulation of the calcium pathway. Our data in rodents and cows indicate that serotonin and calcium are working through a unique feedback loop with PTHrP during lactation to regulate milk calcium and maternal calcium homeostasis.

Key words: calcium, mammary gland, serotonin

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INTRODUCTION

The onset of milk production drains calcium pools in dairy cows due to the initiation of lactation. Colostrum and milk synthesis rapidly depletes calcium from the maternal circulation, and therefore, calcium must be mobilized from maternal bone to maintain adequate circulating concentrations. This is a well-established phenomenon that is critical to the ability of mammals to support their young through lactation (Wysolmerski, 2010; Kovacs, 2017). Circulating calcium concentrations are tightly regu-

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lated by several hormones and metabolites including vitamin D, parathyroid hormone (PTH), calcitonin, and parathyroid hormone-related protein (PTHrP), to name a few. Dietary calcium is insufficient to maintain maternal calcium homeostasis during milk synthesis, requiring alternative mechanisms to achieve calcium adequacy (Wysolmerski, 2010). The mammary gland is the dominant regulator of maternal homeostasis and homeorrhexis during lactation, recruiting nutrient precursors and energy to support milk synthesis (Bauman and Currie, 1980; Hartmann et al., 1998). There has been much research showing that milk synthesis and secretion leads to coordination of maternal metabolism and physiology for lactation after the endocrine environment required to initiate lactogenesis has already occurred. Therefore, it is not surprising that the mammary gland coordinates with bone tissue (a major calcium storage site) through an endocrine feedback loop that is crucial for maternal calcium homeostasis in rodents and humans (Wysolmerski et al., 1995; Wysolmerski, 2010; Goff, 2014). This bone-to-mammary gland feedback system is unique to the physiological state of lactation.

HOMEORHETIC AND HOMEOSTATIC REGULATION OF CALCIUM BY THE MAMMARY GLAND DURING LACTATION

The mammary gland functions as an “accessory parathyroid gland” during lactation, by modulating its own calcium transport into the tissue and simultaneously conserving maternal homeostasis (Fig. 1). The mammary gland produces the hormone PTHrP, which binds to receptors on bone to drive bone resorption and liberate calcium into the systemic circulation (Wysolmerski et al., 1995; Wysolmerski, 2010). The calcium sensing receptor (CaSR) present in the mammary epithelium plays a crucial role in controlling maternal calcium concentrations during lactation. The CaSR is expressed on the basolateral surface of mammary epithelial cells (VanHouten and Wysolmerski, 2013) and, during lactation, has an inverse relationship with the production of PTHrP. Stimulation of the CaSR on normal mammary epithelial cells suppresses production of PTHrP (Ardeshirpour et al., 2006; VanHouten et al., 2007), suggesting a feedback loop whereby sufficient concentrations of blood calcium detected by the CaSR downregulates production of PTHrP. The CaSR is highly expressed in the mammary gland during lactation, compared with virgin and pregnant time periods (VanHouten et al., 2007). In very elegant experiments using transgenic mouse models, it has been repeatedly demonstrated that mammary PTHrP production is responsible for the

mobilization of calcium from the bone during lactation, instead of the typical endocrine regulator of bone PTH (Wysolmerski et al., 1995; VanHouten, 2005; Wysolmerski, 2010, 2012). Disruption of the *PTHrP* gene specifically in mammary epithelial cells in a rodent model resulted in decreased bone turnover and maintenance of bone mass during lactation (VanHouten et al., 2003). The skeletal system maintains its structural and functional roles through communication between 2 cell types, osteoblasts (OB), which are responsible for bone formation, and osteoclasts (OC), which are responsible for bone resorption and, thus, calcium mobilization. Under nonlactating conditions, PTH, in coordination with vitamin D, regulates this endocrine feedback mechanism. Research in humans and rodents suggested that PTH action on bone is uncoupled during lactation (Wysolmerski, 2010; VanHouten and Wysolmerski, 2013). Parathyroid hormone-related protein, like PTH, signals through a common G protein coupled receptor (PTH1R) on the OB to decrease OB cell proliferation and upregulate genes responsible for OC differentiation during lactation. Parathyroid hormone and PTHrP are highly homologous proteins, permitting them to interact with the same receptor (Wysolmerski, 2012). In rodents and humans, increased mammary PTHrP production is responsible for the increased circulating PTHrP during lactation (Thiede, 1994; Wysolmerski et al., 1995; Wysolmerski, 2010; VanHouten and Wysolmerski, 2013).

Therefore, PTHrP, and not PTH, appears to be the critical hormone responsible for induction of bone calcium mobilization during lactation (Wysolmerski et al., 1995). In OB cells, PTHrP increases gene expression of receptor-activated nuclear factor kappa-B ligand (RANKL), matrix metalloproteinase 13 (MMP13), macrophage-colony stimulating factor (M-CSF), IL-6, and monocyte chemotactic protein-1 (MCP1), factors that are known to activate OC cell differentiation (Matsuo and Irie, 2008; Datta and Abou-Samra, 2009). These factors produced by OB cells are then responsible for the induction of numerous genes including *matrix metalloproteinase 9* (MMP9), *receptor-activated nuclear factor kappa-B* (RANK), *cathepsin K*, and *tartrate-resistant alkaline phosphatase* (TRAP; Datta and Abou-Samra, 2009). The OC-induced genes that encode their associated proteins are directly related to the physical degradation of bone through initiation of collagen degradation and mineral dissolution. Therefore, the mammary gland PTHrP and bone OB–OC regulated transcriptional cascade provides the cellular signals needed to increase calcium mobilization from bone during lactation to provide sufficient calcium to support milk formation and maternal calcium homeostasis.

Recent findings from our laboratory showed that serotonin increased PTHrP secretion by the mammary gland in rodent models (Hernandez et al., 2012; Laporta et al., 2014a,b). Furthermore, we demonstrated that serotonin is critical for the expression of various calcium pumps as well as the CaSR in the mammary gland. This review will focus on how serotonin appears to control calcium metabolism in the mammary gland and the potential future directions of our research.

FOUNDATIONAL STUDIES RELATED TO SEROTONIN AND CALCIUM IN RODENTS AND DAIRY COWS

Using a mouse model in which tryptophan hydroxylase 1 (**TPH1**) was selectively deleted in the mammary gland during lactation, we discovered that the mammary gland is a substantial source of serotonin production during lactation (Weaver et al., 2017). Circulating serotonin concentrations are made up of serotonin produced in various tissues in the body, with approximately 90% being derived from the intestines (Ormsbee and Fondacaro, 1985). The synthesis and secretion of serotonin from various non-neuronal tissues allows serotonin to act in autocrine, paracrine, and endocrine manners on the mammary gland during lactation. It was previously established that the mammary gland has 4 to 6 serotonin receptor subtypes, depending on the species (Hernandez et al., 2009; Pai et al., 2009). Dams selectively lacking **TPH1** in mammary tissue during lactation have average circulating serotonin concentrations of approximately 700 ng/mL on d 10 of lactation, whereas their wild-type counterparts average 1,500 ng/mL. Furthermore, when serotonin is overproduced in the mammary gland during lactation, maternal circulating serotonin concentrations increase to approximately 2,000 ng/mL. It therefore appears that serotonin production is not due to alterations in gut serotonin, because duodenal serotonin content does not change with disruption or overexpression of **TPH1**. These results support the hypothesis that serotonin production by the mammary gland is a significant contributor to circulating serotonin concentrations during lactation.

To determine if the relationships between serotonin and calcium observed in rodent models exist in dairy cows, we conducted several preliminary studies. In a small study of 42 multiparous Holstein dairy cows, we observed that serotonin and PTHrP concentrations on d 1 of lactation were positively correlated with total calcium concentrations (Laporta et al., 2013a). Additionally, we observed that serotonin concentrations are dynamic over the course of lactation and decrease around the time of calving (d 0 through 2 of lactation), rebounding

by approximately 10 d into lactation (Moore et al., 2015). The overall average serotonin concentrations in dairy cows are approximately 1,700 ng/mL. However, serotonin concentrations vastly fluctuate, and this is dependent on stage of lactation as well as the individual animal. These results combined with our rodent data provided support for our hypothesis that serotonin and PTHrP are important in the regulation of calcium homeostasis in lactating Holstein dairy cows.

SEROTONIN STIMULATES PARATHYROID HORMONE RELATED-PROTEIN THROUGH COMMUNICATION WITH THE SONIC HEDGEHOG SIGNALING PATHWAY

There is little data in the literature regarding the regulation of PTHrP. One pathway of interest for regulating the production and expression of PTHrP in the mammary gland during lactation is the hedgehog signaling pathway. The hedgehog signaling pathway was originally discovered as a critical pathway for embryonic patterning and development (Wu et al., 2017). Several studies had previously identified hedgehog signaling as an upstream activator of PTHrP synthesis in bone and cartilage development as well as breast cancer (Jemtland et al., 2003; Sterling et al., 2006; Katoh and Katoh, 2009). Additionally, hedgehog signaling was previously shown to be important for mammary gland morphogenesis at the onset of puberty as well as during embryonic mammary gland development (Moraes et al., 2007; Okolowsky et al., 2014). To this end, we explored the possibility that serotonin may modulate PTHrP production through coordination with hedgehog signaling. Interestingly, we determined that **TPH1** knockout mammary glands had little to no expression of sonic hedgehog (**SHH**), its receptor patched 1 (**PTCH1**), downstream target smoothened (**SMO**), or the glioblastoma transcription factors (**GLI1**, **GLI2**, and **GLI3**), which are main components of the pathway (Wu et al., 2017). Upon binding of a hedgehog ligand to its receptor **PTCH1**, **PTCH1** inhibition of **SMO** is alleviated, allowing **SMO** to translocate to primary cilium where it triggers a signaling cascade that targets transcription of **SHH** through **GLI1**. When mice with a **TPH1** disruption were given 5-hydroxytryptophan (**5-HTP**), effectively rescuing circulating serotonin concentrations, expression of the aforementioned hedgehog-associated factors was largely increased. Initially, we were unclear as to how serotonin was stimulating changes in the transcriptional activity of hedgehog target genes. Upon further investigation, we determined that serotonin was acting through a methylation mechanism. We have since identified an early and necessary step in the mechanism of serotonin action on the mam-

mary gland. It reduces methylation of the *Shh* promoter, revealing a novel and efficient alternative start site that drives *Shh* synthesis in the mammary gland, thereby activating mammary hedgehog signaling and synthesis of PTHrP (Fig. 2; Laporta et al., 2014b). It is yet to be determined if this mechanism is also present in the dairy cow, and much remains to be learned about the intricate relationship between serotonin, methylation, and the hedgehog signaling pathway. Additionally, we have some preliminary evidence in mice that serotonin may directly affect methylation of the PTHrP promoter, in addition to its action on the *Shh* promoter. Furthermore, how serotonin achieves this methylation is yet unclear, and we have current work in the laboratory that is focused on better understanding the intricate relationship between serotonin and hedgehog signaling.

SEROTONIN AFFECTS CALCIUM PUMPS IN THE MAMMARY GLAND TO INCREASE MILK CALCIUM CONCENTRATIONS

Calcium sensing, transport, and secretion are impaired in the mammary gland of lactating serotonin-deficient mice. Systemic calcium concentrations are monitored by the CaSR, which is a G protein-coupled receptor present in numerous tissues throughout the body, including the basolateral surface of the mammary epithelium (VanHouten and Wysolmerski, 2013). Mammary CaSR is lowly expressed, except during lactation when expression dramatically increases (VanHouten et al., 2003). In normal mammary epithelial cells, CaSR is inversely related to PTHrP production. This was evidenced by the increased production of PTHrP by the mammary gland of lactating mice harboring a genetic disruption of CaSR (Mamillapalli et al., 2013). Interestingly, it was shown that mRNA and protein abundance of the basolateral CaSR and calcium pump *ORAI1* as well as the apical calcium transporter *PMCA2* were decreased in the mammary glands of TPH1-deficient mice on d 10 of lactation and that intraperitoneal injection of 5-HTP reversed these effects. In addition, serotonin-deficient mice displayed lower circulating and mammary PTHrP concentrations and their femurs had decreased numbers of osteocytes, suggesting an impaired ability to mobilize bone calcium during lactation (Laporta et al., 2014a,b). Additionally, when 5-HTP was supplemented to the diets of rats from d 13 of pregnancy until d 10 of lactation, milk calcium concentrations were increased compared with those of rats fed the control diet (Laporta et al., 2013b). These results suggest that serotonin is critical for the transport of calcium into the mammary gland and milk as well as for the ability of the mammary gland to respond to changes in cir-

culating calcium concentrations. Combined with our previous work demonstrating that PTHrP is decreased in the circulation and mammary glands of TPH1-deficient mice, it is possible to suggest that serotonin's regulation of PTHrP production is through direction of calcium transport into the mammary gland.

To demonstrate the role of serotonin in calcium homeostasis in dairy cows, we performed a preliminary experiment in which varying doses of 5-HTP (0, 0.5, 1.0, or 1.5 mg/kg) were intravenously infused for 1 h to late-lactation dairy cows to determine an optimum dose of 5-HTP necessary to produce significant changes in calcium concentrations. All 3 doses of 5-HTP significantly increased circulating serotonin concentrations (Laporta et al., 2015) to a similar extent in the 2 h after dosing, with concentrations returning to the baseline observed in the saline-infused controls by 2 h after infusion. In addition to serotonin concentration, circulating total calcium concentration was measured. Surprisingly, total calcium concentration and PTHrP concentration decreased in immediate response to 5-HTP treatments (Laporta et al., 2015). To determine the fate of circulating calcium following 5-HTP infusion, we measured urine calcium concentrations prior to the start of infusion and 2 h after the end of the infusion. There was a decrease in urine calcium output with greater doses of 5-HTP treatment, suggesting that calcium was not being lost in urine. When we measured total calcium concentrations in the milk during the infusion periods, the highest dose of 5-HTP increased total milk calcium concentration. Uptake of calcium by the intestine was not measured, but it is possible that there was an increase in intestinal calcium absorption as well. These data support the hypothesis that serotonin causes a transient hypocalcemia, potentially by increased calcium transport into the mammary gland. Increased calcium transport into the mammary gland during lactation is critical for the stimulation of bone calcium mobilization by PTHrP because a transient systemic hypocalcemia is needed for the induction of PTHrP. We need to further explore the underlying mechanisms of how serotonin and mammary gland PTHrP production are regulated in the dairy cow and if they are similar to what we have observed in rodents. It is obvious given our observations in dairy cows and rodents that 5-HTP treatment has differential effects on the calcium and PTHrP system when examining the responses immediately after treatment compared with those after several days of treatment. Therefore, serotonin could improve postpartum calcium homeostasis by initiating the calcium resorption mechanism more rapidly in 2 ways: 1) by directly influencing PTHrP production by the mam-

Figure 1. Calcium movement into the mammary gland regulates maternal calcium homeostasis during lactation. Calcium flux into the mammary gland through the calcium release-activated calcium channel protein 1 (ORAI1) channel increases during lactation to support milk synthesis. Calcium is primarily exported out of the mammary gland in 2 ways: 1) 60% is actively pumped out via the plasma membrane calcium ATPase 2 (PMCA2) transporter on the apical membrane and 2) 40% is bound to milk proteins, primarily casein. Calcium concentrations are sensed by the calcium sensing receptor (CaSR) on the basolateral side of the mammary epithelium. Calcium enters the golgi apparatus through calcium-transporting ATPase type 2C member (SPCA2). Calcium is also stored in the endoplasmic reticulum and is taken up by sarco/endoplasmic reticulum calcium-ATPase (SERCA). As milk calcium concentrations increase and maternal blood calcium concentrations decrease, synthesis and secretion of PTHrP by the mammary epithelium increases. Then, parathyroid hormone related-protein (PTHrP) will bind to the parathyroid hormone 1 receptor (PTH1R) on the osteoblast (OB) cells. This, in turn, increases osteoclast (OC) differentiation and activity and subsequently increases calcium resorption in bone. This occurs through the production of receptor activated nuclear factor kappa B ligand (RANKL) by the OB, which binds to its receptor, receptor activated nuclear factor kappa B (RANK) on the OC. This pathway allows maternal calcium homeostasis to be restored.

mary gland and 2) by increasing calcium transport into the mammary gland and, subsequently, milk.

5-HYDROXYTRYPTOPHAN TREATMENT PREPARTUM IMPROVES CALCIUM HOMEOSTASIS POSTPARTUM, INDEPENDENT OF PARATHYROID HORMONE

After determining that the 1 mg/kg dose of 5-HTP was the optimal concentration for manipulating calcium metabolism in dairy cows, a study was conducted in transition cows to test the ability of using 5-HTP treatment to improve postpartum calcium homeostasis. Dairy cows are most vulnerable to calcium-related pathologies in the days immediately surrounding parturition due to the extreme demands for calcium from the mammary gland. In this study, multiparous Holstein cows received daily intravenous infusions of 1.0 mg/kg of 5-HTP from 7 d before the estimated date of parturition until parturition. Intravenous infusions of 5-HTP prior to parturition increased postpartum total calcium

concentrations compared with saline-treated controls (Weaver et al., 2016). Furthermore, deoxypyridinoline, a marker of OC activity and therefore bone resorption, was measured in the urine. In Holstein cows treated with 5-HTP prepartum, deoxypyridinoline concentrations were increased in the urine on d 1 postpartum. This suggests that Holstein cows treated with 5-HTP have increased bone resorption. These data demonstrate that cows receiving 5-HTP before calving have increased bone resorption at calving. Therefore, 5-HTP treatment before calving may improve postcalving calcium concentrations by increasing bone calcium resorption in multiparous Holstein cows.

The impact of a 5-HTP treatment in Jersey cows, which are known to be very susceptible to hypocalcemia, is also of interest. To examine whether the serotonin-PTHrP-calcium axis is conserved among breeds, Jersey cows 7 d from their estimated date of parturition received 1 mg/kg 5-HTP, alongside the Holstein cows described above. Jersey cows responded to 5-HTP differently than the Holstein cows. Jersey cows infused with 5-HTP had significantly decreased

calcium concentrations prepartum compared with the saline-infused controls and subsequently began to increase calcium concentrations at parturition, whereas the saline-infused controls were decreasing their calcium concentrations at parturition. In fact, control Jersey cows did not reach the nadir for total circulating calcium concentrations until 1 d postpartum (Weaver et al., 2016). Furthermore, Jersey cows treated with 5-HTP had greater concentrations of calcium in their milk compared with the saline-treated cows, which was the opposite of what was observed in Holstein cows. Therefore, serotonin positively impacts calcium homeostasis in both Holstein and Jersey cows, but the underlying mechanisms appear to be different and should be further investigated.

In dairy cows, serotonin concentrations on d 1 of lactation are positively correlated with both calcium and PTHrP concentrations (Laporta et al., 2013a), thereby supporting data in rodents. However, little is known about PTHrP in dairy cows. In a recent study, multiparous cows were administered 1 mg/kg 5-HTP for 1 wk before the estimated date of parturition (Weaver et al., 2016). Circulating calcium concentrations were increased in 5-HTP-treated cows at parturition, corroborating earlier results (Weaver et al., 2016; Hernández-Castellano et al., 2017a,b). Interestingly, PTH concentrations were significantly reduced in the 5-HTP-treated cows compared with the controls (Hernández-Castellano et al., 2017a,b). These data further support the working hypothesis that serotonin is coordinating calcium metabolism during lactation through the PTHrP pathway, rather than the traditional PTH pathway.

DO SEROTONIN, PARATHYROID HORMONE RELATED-PROTEIN, AND CALCIUM FUNCTION IN THEIR OWN FEEDBACK LOOP?

Recent efforts have focused on determining if serotonin is responsible for shuttling calcium into the mammary gland and other tissues during early lactation or if decreased blood calcium concentrations are responsible for increasing serotonin concentrations to help restore calcium homeostasis in the circulation. We performed an experiment in dry, nonlactating dairy cows that were all fed a negative dietary cation-anion difference diet but received 3 different dietary concentrations of calcium (0.45, 1.13, and 2.02%) for 21 d. After the feeding periods were completed, all cows were subjected to a 5% ethylene glycol tetraacetic acid (EGTA) challenge. The objective was to determine how cows responded to induction of a simulated hypocalcemia and how quickly they recovered from the insult. Ionized calcium and serotonin concentrations

were measured every 15 min until cows reached 60% of their initial ionized calcium (Ca^{2+}) concentrations and at 0, 2.5, 5, 10, 15, and 30 min and every 30 min thereafter until 90% of initial Ca^{2+} concentrations were achieved. Preliminary data indicate that cows receiving the 2.02% calcium diet were more resistant to the hypocalcemic challenge, took longer to achieve the 60% target value, and recovered at the same rate as the cows fed the 0.45% calcium diet. Interestingly, the cows consuming the 1.13% calcium diet reached 60% of the target value sooner than other cows. Cows receiving the 0.45% diet reached the 60% target value at the same rate as cows fed the 1.13% calcium diet. These data indicate that cows fed high concentrations of calcium were more resistant to the induction of hypocalcemia and cows fed medium concentrations of calcium had the most dramatic reduction in ionized calcium in response to the induction of hypocalcemia. This is important with regards to how much calcium should be fed in the prepartum diet to potentially prevent dramatic decreases in ionized calcium at parturition. Cows fed the 1.13% calcium diet had the highest concentrations of serotonin compared with the other 2 treatment groups (L. A. Amundson, A. D. Rowson, P. M. Crump, A. P. Prichard, A. A. Cheng, M. Klister, C. E. Wimpler, S. R. Weaver, S. S. Bascom, D. E. Nuzback, K. P. Zanzalari, and L. L. Hernandez, personal communication). The cows receiving the lowest dietary calcium concentration had the lowest serotonin concentrations, and the cows in the high-calcium group were intermediate between the 2 groups during the challenge. Additionally, all serotonin concentrations in these cows were elevated compared with values reported by Moore et al. (2015). These findings suggest that serotonin and calcium are potentially acting in a negative feedback loop to regulate blood calcium homeostasis (Fig. 3). This may indeed be the case in vitro in a bovine mammary epithelial cell model because it was observed that PTHrP mRNA expression was increased by both serotonin and EGTA independently but that the combination of serotonin and EGTA treatment results in the highest expression of PTHrP (L. L. Hernandez and L. A. Amundson, UW-Madison, Madison, WI, personal communication). Further research will be focused on elucidating these mechanisms of action.

CONCLUSIONS

We have demonstrated that serotonin plays a critical role in the regulation of maternal calcium transport, maternal calcium homeostasis, and mammary PTHrP production in rodents. It was also demonstrated that serotonin exerts this regulatory control in part through a methylation mechanism of the sonic hedgehog pathway.

Figure 2. Serotonin directly increases PTHrP synthesis in the mammary gland and indirectly through regulation of the Hedgehog signaling pathway. Serotonin was shown to direct transcription of sonic hedgehog (Shh) in the mammary gland through methylation of Shh transcriptional start sites. The Shh pathway is known to be upstream of parathyroid hormone-related protein (PTHrP). Increased Shh also increases PTHrP, in addition to the direct induction of PTHrP synthesis by serotonin in the mammary epithelium. Serotonin's induction of PTHrP is critical for the release of calcium from bone during lactation. Abbreviations: M = methylation, CaSR = calcium sensing receptor, ORAI1 = calcium release-activated calcium channel protein 1, TSS1 = transcriptional start site 1, TSS2 = transcriptional start site 2, PMCA2 = plasma membrane calcium-ATPase 2.

Figure 3. Serotonin and calcium are working through a feedback loop to modulate maternal calcium homeostasis during lactation. There are 2 potential routes by which serotonin and calcium are used to work to maintain maternal calcium homeostasis during lactation. (A) Increased serotonin concentrations increase calcium flux into the mammary epithelium, by increasing the synthesis of parathyroid hormone-related protein (PTHrP). Increased calcium flux into the mammary gland in turn leads to increases in secretion of calcium into milk. Additionally, increased serotonin concentrations result in increased PTHrP secretion by the mammary gland leading to increased PTHrP action on the parathyroid hormone 1 receptor (PTH1R) on the osteoblasts (OB), to increase osteoclast (OC) activity and differentiation, to restore maternal calcium concentrations. (B) Decreased blood calcium concentrations occur due to increased calcium uptake by the mammary epithelium through calcium release-activated calcium channel protein 1 (ORAI1), resulting in increased secretion of calcium in the milk through plasma membrane calcium ATPase 2 (PMCA2) as well as calcium bound to milk proteins. Decreased calcium concentrations then cause increased serotonin production by the mammary epithelium, leading to increased production of PTHrP. Increased PTHrP in the circulation leads to the increased interaction of PTHrP with the PTH1R on the OB, leading to increased OC activity and differentiation, thereby increasing maternal calcium concentrations through increased bone resorption.

Results with rodent models indicate that the mammary gland is a significant source of serotonin during lactation. Furthermore, observational data in Holstein cows suggest that serotonin, PTHrP, and calcium regulation are interrelated during the early days postpartum, similar to what is observed in rodents. Furthermore, a recent experiment exploring the effects of 5-HTP on maternal calcium homeostasis in late-lactation dairy cows supports the hypothesis that serotonin induces transient hypocalcemia by shuttling calcium into the mammary gland to stimulate mammary production of PTHrP. Increased PTHrP in the circulation during lactation is critical to stimulate the bone calcium resorption necessary to maintain maternal calcium homeostasis. A number of studies indicate that treating Holstein cows with 5-HTP may be used to improve postpartum calcium concentrations. It also appears that Jersey cows respond differently to 5-HTP treatment, and further research should be directed to understanding their physiology compared with that of Holstein cows. Preliminary data examining the interaction between 5-HTP and negative dietary cation–anion difference suggests a synergistic effect of these 2 treatments on increasing postcalving ionized calcium concentrations. Finally, recent data suggest the possibility that serotonin and calcium may be acting in a classic negative feedback loop to maintain blood calcium homeostasis. Together, these findings support the possibility that serotonin is a key player in the search for prevention and treatment of periparturient hypocalcemia in the transition dairy cow.

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