**Making isotonic milk. Focus on “Ca\(^{2+}\)-activated Cl\(^{-}\) channel currents in mammary secretory cells from lactating mouse”**

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The very essence of mammals as a taxonomic class is based on the mammary gland and the ability to provide milk as a total and sole nutritional source at the start of post-partum life. However, much remains to be learned regarding epithelial cellular mechanisms that account for milk composition and volume. In part, this lack of knowledge may reflect the complex interplay of hormones that contribute to gland development during puberty and gestation, to the regulation of activity during lactation, and to the systematic involution of the gland at weaning. Further complicating the scheme is the wide diversity in milk compositions and suckling patterns across the class (6). There is, however, great impetus to fully describe mammary cell function. First, breast feeding is widely practiced in developing countries and there is a reawakening to health benefits associated with breast feeding in developed countries as it may impact health status throughout life. Second, the dairy industry comprises the largest economic segment of animal agriculture in North America and mastitis is the most costly single medical condition in production agriculture. Third, breast cancer ranks first for incidence and second for cause of death in women, with roughly 80% of breast cancers being of epithelial cell origin. Thus, strong arguments can be made on academic, clinical, and economic grounds to develop a greater understanding of mammary epithelial function at the cellular level.

Milk is strictly isotonic and slightly acidic (pH 6.3–6.8) for all species tested. However, composition varies widely across species regarding proteins (<1% wt/vol in humans to >15% in rabbits), fats (<0.5% in rhinoceros to ~50% in seals), and carbohydrates (<0.5% in seals to >12% in wallabies) (6). Mineral composition also varies widely. In general, there is an inverse relationship between fats, proteins, and carbohydrates across species as all are used as energy sources by offspring. Furthermore, the concentration of carbohydrates, typically lactose, varies inversely with monovalent ions, both within a single lactation and across species. Human milk contains (in meq/l) 6–18 Na\(^+\), 12–23 Cl\(^-\), and 11–18 K\(^+\). At the other extreme for which data are readily available, rabbits secrete 60–80 Na\(^+\), 50–60 Cl\(^-\), and 60–80 K\(^+\). Mouse, rat, goat, and cow milk have monovalent ion concentrations that are dispersed over this range. The consistent observation is that milk Na\(^+\) and Cl\(^-\) concentrations are less than those in plasma while milk K\(^+\) concentration is greater. Mechanisms to account for the differences in concentration across the mammary epithelium have not been defined fully.

A theoretical cell scheme to account for ion concentrations in guinea pig milk was proposed in 1971 (5). The representation included Na\(^+\)-K\(^+\)-ATPase in the basolateral membrane with unregulated conductances for Na\(^+\), K\(^+\), and Cl\(^-\) in the apical membrane. A “pump” bringing Cl\(^-\) into the cell was also placed in both the basolateral and apical membranes. It was proposed that Na\(^+\) and K\(^+\) distributed across the apical membrane in accordance with their Nernst potentials leading to the conclusion that basolateral membrane mechanisms including the Na\(^+\)-K\(^+\)-ATPase set the Na\(^+\) and K\(^+\) concentrations in milk. It was immediately obvious that this scheme could not account for diverse milk ion compositions across species and that the scheme could not readily account for the relatively low Cl\(^-\) concentration in milk. Nonetheless, this cellular representation has been reiterated in more recent reviews (9) and has been implicitly incorporated into other theoretical models. It is striking that more than 45 years after the publication of this groundbreaking general cellular model, only incremental progress has been made toward the identification of conductances that account for milk electrolyte composition. It is equally surprising that mechanisms to regulate the proposed conductances, either acutely or chronically, have not been well defined. It was proposed in the 1960s that there was a difference in function between alveolar and duct cells in the mammary gland. The suggestion was that duct cells selectively absorbed Na\(^+\), Cl\(^-\), and water, leaving K\(^+\) at an elevated concentration. Whether this model is correct continues to be debated as little evidence has been gathered to directly test for differences between alveolar and duct epithelia cells.

Kamikawa and coworkers (3), in this issue of *American Journal of Physiology-Cell Physiology*, provide new and solid evidence for the expression of a calcium-activated chloride channel (CaCC), specifically Tmem16a, which is encoded by *Anol*, in the apical membrane of lactating mouse mammary epithelia. The authors provide a thorough assessment, which includes calcium- and voltage-dependent activation, time-dependent activation/inactivation kinetics, anion selectivity, and blocker sensitivity. The biophysical identity has been well described. Importantly, the authors demonstrate that mRNAs for distinct splice variants are expressed and that the protein product(s), as assessed by immunochemistry, is present at or near the apical membrane of epithelial cells lining both alveoli and ducts. In theory, CaCC activity could account for Cl\(^-\) secretion into milk, which might be expected to induce fluid secretion osmotically. The identification of Tmem16a in mammary epithelia provides the opportunity to extend a cell model and to speculate further regarding secretory activity and associated regulatory mechanisms (Fig. 1).

Amiloride-sensitive Na\(^+\) transport has been reported in mammary epithelium, suggesting that the epithelial Na\(^+\) channel, ENaC, might be present. More recently it was shown that ENaC expression or activity was induced by corticosteroids (4, 7, 8). A link between ENaC-mediated ion transport and metastatic transformation has also been suggested. Purinergic agonists are reported to stimulate ENaC-mediated Na\(^+\) absorption via a regulatory cascade that includes mediation by cyto-
components included in this model are the basolateral Na⁺ conductance, ENaC and a Na⁺-K⁺-2Cl⁻ cotransporter via a JAK2/STAT5 pathway to induce Cl⁻ secretion. Is this mechanism active in mammary epithelia in vivo, and if so, what anion channel(s) accounts for the secretion? Likewise, norepinephrine, UTP, and forskolin induce Cl⁻ secretion by mammary epithelia in vitro, all putatively acting via cAMP, and likely via CFTR (1, 2, 8). Are these mechanisms operative in vivo? Importantly, hormone or neurotransmitter-induced Cl⁻ secretion is not conceptually consistent with the relatively low levels of Cl⁻ that are observed in milk. Tmem16a appears to be expressed in both ducts and alveoli (3): is the function consistent throughout the gland? It is tempting to speculate that lactose-associated osmotically induced fluid shifts could change cell volume to activate Tmem16a and thus account for the apparent reciprocal relationship between lactose and electrolyte concentrations, but such speculation must be tested systematically.

Additional transporters including Na⁺/H⁺ exchanger(s), Cl⁻/HCO₃⁻ exchanger(s), and Na⁺-HCO₃⁻ cotransporter have been reported in mammary epithelial cells. These transporters may contribute to pH regulation of milk although their molecular identities, localization, and regulation remain to be determined. Finally, these studies must be broadened to directly address possible differences between alveolar and duct epithelial cells. Ultimately, however, new knowledge gained by Kamikawa et al. (3) and in other recent reports provides avenues to address ongoing developments in mammary physiology as they relate to neonatal nutrition, oncology, and production agriculture.

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REFERENCES


