Regulation of epithelial calcium transport by prolactin: From fish to mammals

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Abstract

Among the reported ~300 biological actions, the established role of prolactin (PRL) is to act as a vertebrate hypercalcemic hormone that regulates epithelial calcium transport in several organs, such as the gills, intestine, and kidney. In fish, PRL stimulates the branchial calcium transport by increasing the activity of Ca\textsuperscript{2+}-ATPase. Although this calciotropic hormone also induces hypercalcemia in amphibians, reptiles and birds, little has been known regarding the underlying mechanism. In contrast, the effects of PRL on the epithelial calcium transport in mammals are well documented. In rodents, PRL has been shown to stimulate the renal tubular calcium reabsorption and intestinal calcium absorption, the latter of which is mediated by the PRL-induced upregulation of calcium transporter gene expression and activities. Recently, we demonstrated that the duodenal calcium absorption in lactating rats was markedly enhanced by the suckling-induced PRL surge, presumably to provide calcium for milk production. The cellular and molecular mechanisms of the PRL-stimulated calcium transport in mammals have been elaborated in this review.

1. Introduction

Prolactin (PRL) has been widely known as an anterior pituitary hormone capable of stimulating milk production in mammals and crop sac development in birds [8,37]. Interestingly, PRL also regulates the transport of ions, such as sodium, calcium, zinc, chloride, and iodide, in many vertebrate species [21,41,64]. By using the immunohistochemical technique, PRL-like immunoreactive signals have been reported in various invertebrate tissues, such as gastro-intestinal tissue of rotifers (Brachionus calyciflorus), cerebral ganglia and reproductive systems of platyhelminthes (Taenia solium and Taenia hydatigena), larvae of nematodes (Trichinella spiralis), and neurons of insects (Apis mellifera) [2,50,63,71]. However, genes of PRL homologs are absent in some invertebrates, e.g., sea urchin, and early chordates, e.g., a cephalochordate Branchiostoma flori\textae\ and a urochordate Ciona intestinalis [36,38]. Therefore, the origin of PRL in metazoan is still controversial, and perhaps the calcium-regulating action of PRL is a vertebrate novelty. In higher chordates, on the other hand, PRL is present in most species, from ray-finned fish to mammals [44], and has a plethora of biological actions pertaining to lactogenesis, maternal behavior, immunomodulation, growth and development, osmoregulation, and epithelial ion transport (for review, see [8]).

Osmoregulatory and ionoregulatory actions of PRL are important for fresh water adaptation in a number of fish, in which it prevents sodium loss through branchial epithelia [53]. PRL also enhances sodium reabsorption in the bladder of fish and amphibians [35,53]. In addition to sodium, the body calcium homeostasis of vertebrates is also under the regulation of PRL, which has been considered as a hypercalcemic hormone in teleost fish, toads, frogs, aquatic amphibians, reptiles, birds, and mammals [8,16,80,82]. It is conceivable that PRL regulates calcium metabolism by modulating calcium mobilization across the epithelia of several organs, e.g., the gill and intestine, as well as calcium release from calcified tissues, e.g., bone (mammals) and scale (fish) [16,32].

2. Theoretical consideration of the epithelial calcium transport

An epithelium is a sheet of cells that line the cavities or surfaces of structures, thus separating the milieu intérieur (e.g., plasma or extracellular fluid) from other compartments (e.g., intestinal lumen, airway, or aquatic environs). In most calcium-permeable epithelia, such as the human and rodent small intestine, calcium traverses the epithelial sheet via the transcellular and paracellular pathways, both of which are tightly regulated by a number of calciotropic hormones, such as 1,25-dihydroxyvitamin D\textsubscript{3} [1,25(OH)\textsubscript{2}D\textsubscript{3}], parathyroid hormone, and PRL.

2.1. Transcellular calcium transport

The transcellular calcium transport is a metabolically energized uphill transport mechanism, which becomes saturated in the presence of high calcium concentration in the apical (luminal) compartment [19,45]. As depicted in Fig. 1A, cellular calcium...
uptake across the apical membrane of polarized epithelial cells, including mammalian intestinal epithelial cells and renal tubular cells, is mediated by the transient receptor potential subfamily V calcium channels (TRPV)-5 or -6, which exhibit a calcium-dependent inactivation [19]. Moreover, the voltage-dependent calcium channels, such as L-type and T-type calcium channels, are also involved in the epithelial calcium uptake [22,52]. Mace and co-workers recently reported that the voltage-dependent L-type calcium channel (Cav)-1.3, which is extremely important for the rapid non-genomic action of PRL in regulating intestinal calcium absorption. Cytoplasmic calcium is buffered by calbindin-D9k and extruded across the basolateral membrane by plasma membrane Ca2+-ATPase (PMCA)-1b and Na+/Ca2+ exchanger (NCX)-1, the latter of which is dependent on the sodium gradient created by Na+/K+-ATPase (NKA). A hypothetical diagram shows the transcellular calcium transport in an osteoclast, which creates a sealing zone to separate the two fluid compartments, i.e., resorptive pit and plasma. Activated osteoclasts normally secrete acid and proteolytic enzymes to dissolve calcified bone. Ionized calcium is transported by TRPV5 across the ruffled membrane into the cytoplasm before being extruded by an unknown transporter.

specifically, in humans and mice, osteoclasts (bone-resorbing cells) but not osteoblasts (bone-forming cells) strongly express TRPV5 transcripts together with the transcripts of other transcellular calcium-transporting proteins i.e., calbindin-D9k, calbindin-D28k, NCX1 and PMCA1b [91]. During the osteoclast-mediated bone resorption, calcium in the resorptive pit is transported by TRPV5 across the ruffled border into the cytoplasm of osteoclasts and extruded into bone extracellular fluid (Fig. 1B), similar to the transport process that occurs in the intestinal cells [91].

2.2. Paracellular calcium transport

Little is currently known regarding the molecular mechanism of the paracellular calcium transport. In general, the epithelial paracellular cellular movement is predominantly driven by electrophysiological gradient and/or solvent drag [11]. Transepithelial calcium (chemical) gradient may occur across the epithelium of gills (calcium in seawater vs. plasma) or intestine (luminal calcium vs. plasma calcium). In the presence of the transepithelial electrical gradient generated by the electrogenic ion transporters, such as Na+/K+-ATPase, calcium passively moves from the fluid compartment with positive potential (e.g., plasma) to that with negative potential (e.g., intestinal lumen) [11]. Na+/K+-ATPase is also essential for the solvent drag-induced calcium transport in the rat small intestinal epithelium, since it pumps sodium into the paracellular space, thereby creating a hyperosmotic microenvironment which, in turn, induces lumen-to-plasma vectorial water movement. Thus, ionized calcium in the luminal fluid can move to the basolateral side along the paracellular pathway in the stream of water—a process known as solvent drag [85]. However, the paracellular calcium transport is not a simple passive process, but is indeed regulated by the tight junction proteins of the claudin family. Some claudins, such as claudin-2 and -12, have been hypothesized to form calcium-permeable pores in the tight junction, and may allow the paracellular calcium transport to occur in a channel-like manner [33]. In other words, the tight junction exhibits size and charge selectivity for the paracellular ion movement, and the overexpression of certain claudins may profoundly affect the epithelial permeability [3]. For example, during freshwater acclimation of euryhaline Atlantic salmon, an increase in claudin-30 expression in the branchial epithelium may help increase the tightness of the gill by decreasing the epithelial

Fig. 1. (A) A diagram shows transepithelial calcium transport in the rat duodenum. It is hypothesized that, between meals when luminal glucose is low, the apical calcium uptake is predominantly mediated by the transient receptor potential subfamily V (TRPV)-5/6 calcium channels, which open in the presence of hyperpolarized apical membrane potential (resting potential –48 mV). When the luminal glucose concentration is increased, glucose is transported via sodium-dependent glucose transporter (SGLT)-1 together with sodium, thereby depolarizing the apical membrane. This depolarizing process (dashed box) then triggers opening of the voltage-dependent L-type calcium channel (Cav)-1.3, which is extremely important for the rapid non-genomic action of PRL in regulating intestinal calcium absorption. Cytoplasmic calcium is buffered by calbindin-D9k, and extruded across the basolateral membrane by plasma membrane Ca2+-ATPase (PMCA)-1b and Na+/Ca2+ exchanger (NCX)-1, the latter of which is dependent on the sodium gradient created by Na+/K+-ATPase (NKA). (B) A hypothetical diagram shows the transcellular calcium transport in an osteoclast, which creates a sealing zone to separate the two fluid compartments, i.e., resorptive pit and plasma. Activated osteoclasts normally secrete acid and proteolytic enzymes to dissolve calcified bone. Ionized calcium is transported by TRPV5 across the ruffled membrane into the cytoplasm before being extruded by an unknown transporter.
permeability to cations [26]. Intestinal expressions of some claudins have been reported to be 1,25(OH)2D3-dependent [20,33]. Specifically, 1,25(OH)2D3 was found to upregulate the expression of claudin-2 and -12 [33], while downregulating the expression of claudin-3 [49].

3. PRL and epithelial calcium transport

3.1. Fish

In contrast to the terrestrial vertebrates, marine and freshwater vertebrates live in calcium-rich environments. Marine fish usually have an intermediate level of plasma calcium between that in seawater and mammalian plasma [56]. The fish intestine absorbs ionized calcium into the body, which is later stored in bone (only in bony fish with calcified bone) and scales or excreted by the kidney [32]. Even in chondrichthyan fish, calcium is required for maintaining the strength of the cartilaginous skeletons [23,56]. Because of a very large surface area and direct exposure to the surrounding water, calcium uptake by the branchial epithelium also contributes to the body calcium homeostasis [32]. In euryhaline tilapia Oreochromis mossambicus, the branchial chloride cells were found to mediate calcium influx, which depended on the activities of PMCA and NCX [30]. Since PRL receptors have been identified in the gills and intestine of several species, including freshwater-adapted O. mossambicus [24,62], both organs may be the important targets of the hypercalcemic action of PRL.

The hypercalcemic action of PRL is closely related to its osmoregulatory action. In euryhaline teleosts, a seawater-to-freshwater transfer markedly increases the plasma levels of PRL that, in turn, helps restrict sodium loss [4]. Concurrently, PRL also enhances the branchial absorption of calcium from the surrounding freshwater that has relatively low calcium as compared to seawater. However, in the absence of salinity challenge, a change in ambient calcium concentration alone either in freshwater- or seawater-adapted fish seems to be less potent in stimulating PRL secretion [4]. Seale and co-workers suggested that PRL-producing cells were sensitive to changes in plasma osmolality [72]. A decrease in the extracellular osmolality can enhance PRL release by opening the stretch-activated ion channels, which allow extracellular calcium ions to enter the swollen PRL-producing cells [72].

Flik and co-workers demonstrated in freshwater-acclimated North American eel (Anguilla rostrata) that ovine PRL injection and hyperprolactinemia induced by the rostral pars distalis transplanted significantly elevated plasma calcium concentrations by enhancing the maximal velocity of PMCA in the branchial epithelial cells [29]. In male tilapia O. mossambicus, an 8-day PRL administration increased the gill calcium influx while decreasing calcium efflux, leading to hypercalcemia [31]. Although PRL markedly enhances the branchial calcium transport, whether PRL stimulates intestinal calcium absorption in fish remains controversial. It is believed that the fish intestinal calcium transport is under the control of stanniocalcin, a hypocalcemic hormone released from the corpuscles of Stannius [32]. However, PRL was recently found to downregulate the expression of cation-selective claudin-15 in the pyloric caeca and mid-intestine of Atlantic salmon [89]. Since claudin-15 is essential for the PRL-enhanced intestinal calcium transport in human intestinal-like Caco-2 monolayer [14], PRL might modulate the intestinal paracellular calcium transport in fish by altering claudin-15 expression.

Interestingly, PRL may promote calcium accretion in fish bones and scales. Takahashi and co-workers demonstrated in the scales of female goldfish (Carassius auratus) that PRL could induce osteoclast apoptosis, thereby reducing osteoclast activity and calcium loss from the scales [84].

3.2. Amphibians

The amphibian uptake of calcium occurs across the skin and small intestine, especially in the duodenum [80], while the gills are also capable of transporting calcium from the surrounding water in anuran tadpoles [6]. Subsequently, calcium is stored in calcified bone or as calcium carbonate crystals in the paravertebral lime-sacs or endolymphatic sacs [80]. The endolymphatic calcium carbonate crystals are formed by otoconin-22 protein, the expression of which is upregulated by the hypocalcemic hormone calcitonin [70,95]. In addition, the plasma calcium ions that are filtered through the kidney can be reabsorbed transcellularly by the renal tubular cells and urinary bladder [80].

PRL has been reported to induce hypercalcemic responses in both tadpoles and adult Rana catesbeiana [5,68], but the underlying cellular mechanism remains elusive. On the other hand, male hypophysectomized aquatic salamanders (Necturus maculosus) manifested hypocalcemia, which could be alleviated by ovine PRL administration [58]. PRL receptor immunoreactivity has also been observed in the frog epidermis, in which PRL provides morphological and functional integrity of skin, and also promotes the epithelial Na+ channel (ENaC)-mediated transcellular sodium transport across the skin [73,83]. However, the effect of PRL on calcium transport across the amphibian skin epithelium requires future investigation.

3.3. Reptiles

Similar to amphibians, the major sites for endocrine regulation of reptilian calcium metabolism are kidney, bone and endolymphatic sacs [56], and evidence pertaining to the role of PRL on epithelial calcium transport is elusive. Pituitary PRL transcripts have been identified in various reptilian species, e.g., sea turtle (Chelonia mydas), alligator (Alligator mississippiensis) and crocodile (Crocodylus novaeguineae) [43,57,96]. Recently, PRL mRNA expression has been reported in the extrapituitary tissues of leopard gecko (Eublepharis macularius), e.g., ovary and testis. PRL receptors have been identified in a number of reptilian tissues, such as heart, lung, liver, kidney, adrenal gland, ovary, oviduct, testis, stomach, and small and large intestine [43]. Moreover, Cheng and co-workers reported the presence of PRL receptor in the kidney and large intestine of the common rat snake (Ptyas mucosus), as determined by 125I-PRL binding assay [18].

The effect of PRL on calcium metabolism has long been studied in reptiles, but the cellular mechanism is not well understood. PRL administration progressively induced hypercalcemia in a freshwater snake (Natrix piscator) and garden lizard (Calotes versicolor) [77,78]. Swarup and co-workers also showed that daily intraperitoneal injection of ovine PRL for 7 days significantly elevated the serum calcium levels in Varanus flavescens [82]. However, it is still not known whether PRL induces hypercalcemia in reptiles by enhancing intestinal calcium absorption or calcium release from bone or endolymphatic sacs.

3.4. Birds

Unlike lower vertebrates, avian PRL functions toward reproduction and parental behavior, similar to that observed in mammals [25,97]. Interestingly, in pigeons and doves, PRL enhances the growth and development of the epithelium lining the crop sac—a expansion of the lower esophagus—as well as production of protein- and lipid-rich cropmilk [37]. Although cropmilk also contains calcium, and PRL can induce the expression of the cropmilk calcium-binding protein annexin I [37], it is not known whether PRL stimulates calcium transport across the crop sac epithelium. A recent microarray study in King pigeons (Columbia livia) revealed that
the PMCA3 mRNA level in the cropmilk-producing crop sac was
~0.06-fold compared to that in normal crop sac [34], suggesting
that PMCA3 may not contribute to the crop sac calcium transport.

Regarding the avian calcium homeostasis, the intestine, bone and kidney act in concert to maintain normocalcemia, similar to reptiles and mammals [56]. PRL is also a hypercalcemic hormone in birds, such as C. livia [79]. It has been hypothesized that PRL indirectly stimulates the avian intestinal calcium absorption through 1,25(OH)2D3, since PRL can increase the activity of 1α-hydroxylase [75], the rate-limiting enzyme for 1,25(OH)2D3 production. Spanos and co-workers demonstrated that male chicks subjected to daily injection of PRL for 5 days exhibited a 2-fold increase in the plasma 1,25(OH)2D3 levels [76]. In chickens, 1,25(OH)2D3 is the major calcitropic hormone that stimulates intestinal calbindin-D28k and PMCA expression, thereby increasing the rate of calcium absorption [9,46,94].

Although PRL has been shown to exert its osmoregulatory action by increasing the urine flow rate and fractional excretion of sodium and chloride [65,66], it did not alter the fractional excretion of calcium, at least in European starlings (Sturnus vulgaris) [66]. PRL might, therefore, induce hypercalcemia by enhancing the intestinal calcium absorption and/or bone resorption. Nevertheless, the renal tubular cells should fine-tune their function to reabsorb the large amount of filtered calcium during the PRL-induced hypercalcemia; otherwise, overt hypercalcuria may occur.

Calcium demand is markedly increased in laying birds since the uterine epithelium transfers a huge amount of calcium for eggshell formation within hours [7,60]. The eggshell is composed of calcium salts supported by a protein matrix. Previous investigations in female Japanese quail (Coturnix japonica) showed that the transepithelial calcium flux in the uterine mucosa was an ATP-dependent active process that required Na+/K+-ATPase and carbonic anhydrase, but not PMCA [59,60]. Coincidentally, the putitary production and secretion of PRL are markedly increased in laying birds, such as geese and turkeys [69,97]. Although this hyperprolactinemia is primarily essential for the incubation behavior [25], it is tempting to speculate that PRL could play an important role in regulating the uterine epithelial calcium transport in laying birds.

3.5. Mammals

Similar other vertebrates, PRL administration also induces hypercalcemia in mammals, at least in mice [1]. Since PRL receptors have been identified in the rodent intestinal epithelial cells, osteoblasts and renal tubular cells, these cells are potential targets of the calcitropic actions of PRL [15,39,67,74]. Our laboratory has demonstrated that PRL directly and rapidly (within 8 min) stimulates the duodenal calcium absorption in female rats in an 1,25(OH)2D3-independent manner [12,13]. This rapid action of PRL is non-genomic and dependent on various intracellular signaling mediators, such as phosphoinositide 3-kinase (PI3K), protein kinase Cα and RhoA-associated coiled-coil forming kinase (ROCK) [39,86,87]. At the cellular level, PRL rapidly enhances the apical calcium uptake via Cαα1.3, rather than TRPV5/6 channels [54]. It also promotes the activities of PMCA and Na+/K+ -ATPase, thereby increasing the transcellular and solvent drag-induced calcium transport across the duodenal epithelium, respectively [13,85]. Moreover, the PRL-enhanced paracellular passive calcium transport in human Caco-2 intestinal cell monolayer results from the serine phosphorylation of claudin-15, while siRNA against claudin-15 can abolish such the PRL effect [14].

On the other hand, long-term exposure to PRL induces a transcriptome response in the duodenal cells, in part, by upregulating the expression of TRPV5/6, calbindin-D28k and PMCA1b, leading to a sustained increase in the duodenal calcium absorption [14,17,90]. Ajibade and co-workers further provided evidence that

PRL induced a 4-fold increase in TRPV6 mRNA level, and cooperated with 1,25(OH)2D3 in regulating TRPV6 and calbindin-D28k expression in the mouse duodenum [1].

Besides the small intestine, a considerable magnitude of trans-epithelial calcium transport also occurs in the large intestine, particularly in the cecum that has the highest rate of calcium absorption as compared to other intestinal segments of rats [42]. We recently reported that the cecal calcium transport was important for body calcium homeostasis since the cecectomy rats manifested fecal calcium wasting, negative calcium balance and widespread bone loss [40]. Interestingly, the rat cecum also responds to PRL by increasing the active and passive calcium transport [48], consistent with that observed in the duodenum.

In pregnant and lactating rats, physiological hyperprolactinemia markedly increases the duodenal calcium absorption in a two-step manner [14,16]. Specifically, a prolonged exposure to 100–300 ng/mL PRL (normal non-pregnant levels ~7–10 ng/mL) during these reproductive periods upregulates the expression of calcium transporter genes, leading to an elevation of the calcium transport baseline—known as the step-1 calcium transport. In lactating rats, the suckling-induced PRL surge (up to 800 ng/mL) further increases calcium flux to the step-2 level above the newly elevated baseline (step-1) [14,16]. It is hypothesized that the step-2 calcium transport provides additional calcium to match calcium loss in milk during breastfeeding.

Regarding other calcium-regulating organs, PRL injection was found to decrease the renal calcium excretion in both non-pregnant and pregnant rats [51,61], suggesting that PRL also regulates the renal tubular calcium transport. In lactating rats, PRL stimulates the osteoclast-mediated bone resorption, presumably to provide ionized calcium for milk production [81]. Little has been known whether PRL is capable of enhancing the placental and mammary calcium transfer. However, in humans and cows, PRL might indirectly control milk calcium levels by regulating the synthesis of casein, a calcium-binding protein in milk [55].

4. Concluding remarks and perspectives

Although the hypercalcemic action of PRL is evident in vertebrates from fish to mammals, its physiological role in regulating epithelial calcium transport, especially in the calcium-regulating organs, such as intestine and kidney, is not well understood. Indeed, the effects of PRL on the transepithelial calcium flux have been widely investigated only in fish and mammals. In bony fish, PRL enhances calcium transport in the gills of both freshwater and euryhaline species, leading to hypercalcemia. In mammals, PRL stimulates calcium absorption in the small and large intestine, especially in the duodenum and cecum, by upregulating the expression and activities of several calcium transporter genes, such as TRPV6, calbindin-D28k and PMCA1b. PRL becomes more important for lactating mammals since it elevates the baseline of intestinal calcium absorption, and markedly and rapidly increases calcium uptake during the suckling-induced PRL surge. If the intestinal calcium supply is inadequate, PRL can increase bone resorption to provide ionized calcium for milk production. Besides the intestine, PRL also increases the renal calcium reabsorption, thereby reducing the urinary calcium loss.

Although the PRL gene has been postulated to be a vertebrate innovation, the PRL-like immunoreactive signals were previously reported in a number of invertebrate species. It is still an open question whether invertebrate PRL-like protein regulates epithelial transport of calcium or other ions. Furthermore, more studies are required to demonstrate experimentally the direct effects of PRL on the intestinal and renal calcium transport in fish, amphibians and reptiles, as well as on the crop sac calcium transport in pigeons.
Even in mammals, how PRL regulates calcium transport across the renal tubular epithelium, mammary epithelium, and placenta remains to be investigated.

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**References**


