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REVIEW

Hormonal Regulation and Implication of Cell Signaling in Calcium Transfer by Placenta

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During pregnancy, the human placenta transfers about 30 g of calcium (Ca^{2+}) from the mother to the fetus. This transfer is mainly done during the third trimester, at a rate of 140 mg/(kg·d). It allows adequate growth and development of the fetus, since Ca^{2+} is vital for the mineralization of the fetus's skeleton and many cellular functions. Because Ca^{2+} flows through the placenta against an electrochemical gradient, calcemic hormones could also be involved to overcome this gradient. Hormones such as calcitonin, parathyroid hormone (PTH), and PTH-related peptide (PTHrP) have been found in maternal and fetal circulation, and they originate from both parties, as well as from the placenta in the case of PTHrP. As the placenta possesses most of the G-protein-coupled receptors to bind these hormones, it is likely that they play an important role in maternal-fetal Ca^{2+} homeostasis. More studies are needed to assess the importance of these hormones in the regulation of Ca^{2+} management during pregnancy, and to understand better the cell-signaling pathways involved. This article addresses the current knowledge in this field to guide future investigations on the roles, functions, and localizations of the components involved during Ca^{2+} transfer by syncytiotrophoblasts.

Key Words: Placenta; calcium; PTHrP; PTH, signaling.

Calcium: Essential Element

During pregnancy, adequate mineralization of the skeleton and development of the fetus rely on three closely related parameters: the mother's adequate food intake, the function of the placental unit, and the ability of the fetus to use these supplies *(1)*. One third of the unidirectional maternofetal Ca^{2+} transfer is owing to transcellular saturable components *(2)*. Thus, the demands of fetal growth lead to an adaptation of maternal homeostasis in order to provide all the required Ca^{2+} (130–150 mg/[kg·d]) *(3)* from enhanced intestinal absorption rather than from mobilization of maternal skeletal reserves, which could cause severe problems. The mammalian fetus is maintained hypercalcemic relative to its mother, in part by an action of a placental Ca^{2+} pump located on the basal plasma membrane (BPM) of the syncytiotrophoblast *(4,5)*. From the wk 28 of pregnancy until term, as fetal weight triples, the Ca^{2+} content increases fourfold and bone mineral density increases progressively *(6)*. Several factors are known to be involved in maintaining the relationship between the large pool of Ca^{2+} in the skeleton and the much smaller pool in the extracellular fluid. Besides Ca^{2+} itself, other related nutrients such as magnesium (Mg^{2+}) and phosphate and hormones such as calcitriol, parathyroid hormone (PTH), PTH-related peptide (PTHrP), and calcitonin are among those factors *(7–9)*. This transfer is done through the syncytiotrophoblast, which is a physical and a selective barrier for various nutrients. It consists of bipolar syncytiotrophoblast cells formed with two distinct membranes: one is a brush-border membrane (BBM) facing the maternal circulation, and the other is a basal plasma membrane (BPM) facing the fetal circulation.

The polarity of the syncytiotrophoblast structure implies particularities in the transfer of Ca^{2+} through the placenta. By their microvillosities, BBMs provide a wider surface to facilitate absorption of nutrients. Since the concentration of Ca^{2+} is low in the cytosol of syncytiotrophoblast cells (0.1–1 μ*M*) *(10)*, in comparison with the maternal one (2.13 m*M*) *(11)*, Ca^{2+} crosses the BBM mostly by different types of passive mechanisms *(12,13)*. However, the presence of Na^{+}/Ca^{2+} exchangers in BBM have been demonstrated *(14)*.

In many cells, Ca^{2+} is transported by calcium binding proteins (CaBP). In the placenta, proteins from this large family have been identified, including oncomodulin *(15)* and S100-P in humans *(16)*. Recently, a 57-kDa calbindin has been identified in mouse trophoblast *(17)*. On the other hand, localization of a 9-kDa calbindin is still controversial, regarding species *(18–20)*. These shuttles provide a buffer for cytosolic Ca^{2+} concentration, so that intracellular processes are not altered.

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Confronted with the high concentration of Ca^{2+} in the fetus's blood, energy-consuming mechanisms and probably hormone-related mechanisms must be privileged. Indeed, Lafond et al. *(4)* have shown the existence of two different Ca^{2+} transport mechanisms in the BPM: first, an adenosine triphosphate (ATP)-dependent Ca^{2+} transport and, second, a basal Ca^{2+} transport. The latter can be inhibited by Mg^{2+} and saturated. Since the ATP-dependent Ca^{2+} transport requires the presence of Mg^{2+}, it suggests the implication of a Ca^{2+}/Mg^{2+}-ATPase located in BPM *(4)*. Once in the fetal circulation, Ca^{2+} is used especially by the fetus for bone mineralization, but also for hormone secretion, enzymatic functions, cell signaling, muscular contraction, and many other metabolic processes.

More than 99% of Ca^{2+} is stored in teeth and bones. In the latter tissue, Ca^{2+} is responsible for structural strengh and serves as storage in case of the need to maintain Ca^{2+} homeostatsis *(21)*. Also, high levels of Ca^{2+} inhibit osteoclastic bone resorption *(22)*. In the kidney, a high concentration of Ca^{2+} inhibits 1-hydroxylation of 25-hydroxyvitamin D, tubular reabsorption of NaCl, Ca^{2+}, and Mg^{2+} in cortical ascending limb (for a review see ref. *23*). It also inhibits the accumulation of hormone-stimulated cyclic adenosine monophosphate (cAMP) *(24)* and the effects of antidiuretic hormone *(25)*. Futhermore, Ca^{2+} is involved via a calcium-sensing receptor in nonhomeostatic processes in cells and organs such as the brain *(26)*, cytotrophoblasts *(27)*, keratinocytes *(28)*, and lens epithelial cells *(29)*.

Proliferation and differentiation are important phenomena in fetal development, and many cells depend on Ca^{2+} to accomplish these tasks. In fact, human T-cells differentiate into type 1 or type 2 according to the balance between Ca^{2+} signaling and protein kinase C (PKC) activity *(30)*. Keratinocytes *(28)*, murine neuronal crest *(31)* immature dendritic cells (myeloid cells) *(32)*, and osteoblast cells *(33)* are only a few examples of other cells requiring Ca^{2+} for differentiation or proliferation. In most contractile cells, extracellular Ca^{2+} and Ca^{2+} from intracellular stores play a crucial role at many levels in the contraction process. During early development, oscillation of intracellular Ca^{2+} concentration in cardiomyocytes is responsible for small depolarization, as well as spontaneous contractions even before the rythmic beating of the heart has manifested itself *(34)*.

Furthermore, Ca^{2+} can act as a first- or second-messenger link to the secretion of calcemic hormones such as calcitonin *(35)* and PTH *(36,37)*, as well as other hormones and enzymes such as insulin *(38)*, amylase from rat pancreas *(39)*, histamine from enterochromaffin-like cells *(40)*, and neuropeptide Y in placental trophoblasts *(41)*. Plasma renin activity and aldosterone secretion are modulated by serum-ionized Ca^{2+} in human subjects *(42)*. It has also been shown that altered placental and renal handling in rat diabetic pregnancy could reflect an altered production or function of Ca^{2+} regulatory hormones *(43)*. Thus, Ca^{2+} is a crucial ion in the adequate growth and development of the fetus, as well as throughout the fetus's life-span.

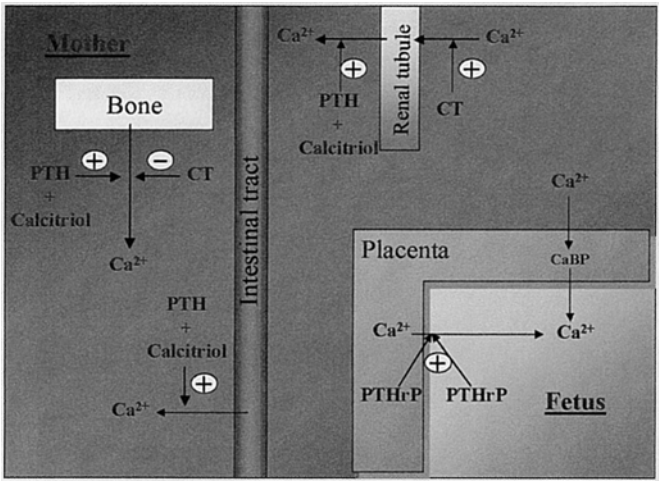

Fig. 1. Effects of PTH, PTHrP, and calcitonin on calcemia in placental, bone renal, and intestinal models.

Major Calciotropic Hormones

PTH and PTHrP

PTH is secreted by the parathyroid gland of the mother and her fetus. It plays a vital role in Ca^{2+} homeostasis. It also acts on low blood Ca^{2+} levels by enhancing bone resorption and inhibiting renal excretion of this ion, as seen in Fig. 1. In this view, PTH could also exert an effect on fetal calcemia by an action on placental function.

As for PTHrP, in 1987 it was first found in patients with humoral hypercalcemia of malignancy, causing a modification in the Ca^{2+} concentration in serum and a suppression of the PTH levels, but it did not impair phosphatemia *(44–46)*. PTHrP is also an oncofetal hormone. Early in gestation and until term, it is produced by the uteroplacental unit *(47)*. At wk 9 postconception, the developed fetal parathyroid glands are also able to synthesize and release PTHrP into fetal circulation *(48,49)*. It seems that PTHrP is important in the Ca^{2+} transfer from the mother to her fetus *(50,51)*. PTHrP shares 8 of the first 13 amino acids in the N-terminal region of PTH, creating a great degree of homology between the two peptides and supporting the fact that PTHrP can mimic the effects of PTH on kidney and bones *(52)*. PTHrP is expressed and secreted by a variety of normal fetal and adult tissues as well as pathologic tissues. Briefly, PTHrP has been located in the fetal central and peripheral nervous systems, in salivary and adrenal glands, in liver, in lung, throughout the urogenital and musculoskeletal systems, in amniotic membranes, and in many other fetal tissues *(53)*. In addition, amniotic fluid contains measurable quantities of PTHrP (40 pmol/L, at term) *(54)* that could exert paracrine effects on fetal gastrointestinal pulmonary cells and skin since the fetus swallows, inspires, and baths in amniotic fluid.

Many studies support the role of PTHrP in placental Ca^{2+} transport. Figure 1 summarizes the effects of this hormone in the placenta, as well as in other systems. It could therefore be involved in maintaining the maternofetal Ca^{2+} gradient. In fact, Care et al. *(55)* have demonstrated that PTHrP (fragment 67–86) corresponds to the portion of the peptide that is most likely responsible for simultaneously stimulating placental Ca^{2+} and Mg^{2+} transport. On the other hand, Barlet et al. *(56)* have shown that PTHrP (1–34) stimulates Ca^{2+} transfer from the mother to her fetus. Using a sheep placental perfusion model, Rodda and Mosely *(44)* showed that PTHrP increases placental Ca^{2+} transfer from mother to the fetus, when an equivalent concentration of PTH is not effective. Moreover, a fetal thyroparathyroidectomy at the midgestation of the sheep results in an inversion of the fetal-maternal Ca^{2+} gradient as well as considerable fetal skeletal demineralization *(57)*, showing the importance of the fetal PTHrP in the maintenance of this fetal-maternal Ca^{2+} gradient *(44)*. Kovacs et al. *(58)* have demonstrated that in homozygous fetal mice in which the PTHrP gene or PTH/PTHrP receptor has been ablated, ionized Ca^{2+} was significantly lower than in heterozygous or wild-type littermates. They also showed that in this situation the transplacental Ca^{2+} transport is reduced and the maternal-fetal Ca^{2+} gradient is reversed. Furthermore, PTHrP seems to regulate, in this case, Ca^{2+} transport from mother to her fetus by a specific receptor other than the PTH/PTHrP receptor, which remains to be identified *(58)*. Furthermore, knockout of the PTHrP gene is lethal in transgenic mice. Neonatal mice die shortly after birth and show many skeletal defects, illustrating the critical importance of PTHrP in normal fetal growth and development *(59)*. Tucci et al. *(60)* have obtained similar results, but the circulating total Ca^{2+} levels of the fetal homozygous mice in which the PTHrP gene was ablated did not differ from the heterozygous or wild-type littermates. The total-body Ca^{2+} levels of the mutants were also higher than those of the normal littermates. They concluded that the role of PTHrP in maintaining the integrity of the transplacental Ca^{2+} pump remains unclear. In this view, they proposed a nonuniversal mechanism of the action of PTHrP and hypothesize that while PTHrP acts directly on the placenta to achieve Ca^{2+} transfer from the mother to fetus in sheep, it could be implicated in other species, such as rodents, to act in a paracrine way in fetal circulation.

Calcitonin

Calcitonin has a hypocalcemic effect among mammals *(66)* mainly in renal tubular cells *(62)* and bone cells *(63)*, as summarized in Fig. 1. Its concentration is highest at the fetal level *(6)*, and a linear relationship exists between its rate of hormonal secretion and the fetal plasma Ca^{2+} concentration *(64)*. Nevertheless, it seems that calcitonin does not affect or has little effect *(65)* on fetal calcemia and parathormonemia. By contrast, it is known that under pharmacologic conditions, calcitonin induces maternal hypocalcemia. Barlet *(7)* has demonstrated that a calcitonin deficiency among pregnant ewes is associated with an increase in the Ca^{2+} transfer from the mother to fetus. It is known that during pregnancy, the production of maternal calcitonin in ewes, and perhaps other species, protects the mother from demineralization of her own skeleton *(66)*.

Vitamin D_3 (1,25[OH]$_2D_3$) or Calcitriol

$1,25(OH)_2D_3$ or calcitriol is involved in the mother's calcemic homeostatic process because it is the main regulator of intestinal absorption of Ca^{2+} and acts, in concert with PTH, on renal absorption and bone mineralization *(67)* (Fig. 1). Calcitriol also regulates the synthesis of various calbindins (CaBP), which are intracellular proteins implicated in the Ca^{2+} homeostasis of various cells. This type of regulation applies to CaBP such as the renal 28-kDa CaBP *(68)* and the 9-kDa intestinal CaBP *(69)*. Maternal concentration of $1,25(OH)_2D_3$ increases throughout gestation, mainly during the third trimester, and could be linked to the required increase in fetal Ca^{2+} during this period *(70)*. It is well known that even though $1,25(OH)_2D_3$ passes through the human placenta, its fetal level is relatively low *(71)*. Garel *(72)* demonstrated the real hypercalcemic effect of this hormone in rat and sheep fetuses, but it is not known whether this effect is related to an action on fetal bone, kidney, or placenta. Moreover, rat dams that are severely deficient in $1,25(OH)_2D_3$ and are hypocalcemic have fetuses with normal Ca^{2+} levels and totally mineralized skeleton *(73)*. Other hormones could be involved in the calcemic homeostasis process since Ardawi et al. *(71)* have demonstrated a positive correlation between the previously mentioned calcitriol and PTHrP, both of which increase throughout gestation. Jones et al. *(74)* have shown that because the final active metabolite of calcitriol the final active metabolite of calcitriol [$1,25(OH)_2D_3$] is synthesized in the renal proximal tubules, in nephrectomized sheep fetus the calcitriol concentration is reduced and the fetus becomes hypocalcemic. This conclusion was made after they observed a reduction in the Ca^{2+} uptake into the maternal-fetal-facing chorion. However, the addition of calcitriol rapidly increased the rate of Ca^{2+} uptake. They observed that the placental barrier was, at least partially, under the control of calcitriol. Furthermore, the direct or indirect implication of $1,25(OH)_2D_3$ in the modulation of the placental Ca^{2+} flux could be plausible because the placenta synthesizes this vitamin *(5)*.

Signal Transductors in the Placenta

G-Proteins

Briefly, the heterotrimeric G proteins consist of three different subunits, α, β, and γ *(75,76)*, that dissociate in an

α-subunit and a βγ dimer when guanosine 5'-triphosphate (GTP) binds to α-subunits. Then, the free α-GTP subunit can modulate the activity of various effectors *(77–78)*, because the activity of several enzymatic systems can also be directly or indirectly modulated by the βγ complex *(76,80–83)*. The four members of the α_s-subunit family of the G-proteins are adenosine 5'-diphosphate (ADP) ribosylable and sensitive to *Vibrio cholerae* toxin *(84)*. They represent the regulative subunit that couples the receptor to the adenylyl cyclase (AC) activation *(85)* and modulate the Ca^{2+} channels sensitive to dihydropyridines *(86)*. The three α_i-subunits of G-proteins are also ADP ribosylable, sensitive to *Bordetella pertussis* toxin (*PTX*), represent the regulative complex inhibiting the AC *(87)*, and appear to modulate the activity of K^+ channels *(88,89)*. The four α_o-subunits of G-proteins *(90)* have several analogies with the α_i-subunits, since they are ribosylable by *PTX (91,92)*, modulate the activity of some voltage-dependent Ca^{2+} channels (VDCCs) *(93,94)*, and do not appear to be coupled to phospholipase C (PLC) *(95)*. Furthermore, they could be involved in the opening of K^+ channels *(96)* and the inhibition of Ca^{2+} channels voltage independent and insensitive to dihydropyridine. Other α-subunits of G-proteins have been characterized, such as the α_z-subunit, which regulates the K^+ and Ca^{2+} channels *(75,97)*, and the α_q subunit, which activates PLC *(97)*. On the other hand, βγ dimer assumes four major functions:

1. Maintaining the α-subunit inactive by suppressing the liberation of guanosine 5'-diphosphate.
2. Ensuring the high-affinity association with the receptor.
3. Acting as a link to the cytoplasmic membrane.
4. Modulating various effectors such as certain AC, Ca^{2+}, and potassium channels *(77)*.

In the placenta, a recent study demonstrated the presence of several α-subunits (G_{il}, G_{i3}, G_s, G_q, G_z, and G_o) and a β-subunit of G-proteins asymmetrically distributed in the BBM and BPM of the syncytiotrophoblast *(98)*. Previously, Evans et al. *(99)* showed the presence of a β-subunit in placental homogenate. Petit et al. *(100)* also demonstrated an important variation in the expression of some G-proteins (G_{i2} and G_{i3}) in human placenta in hydatiform moles suggesting alterations in the signal transduction machinery within molar trophoblasts. Therefore, to have a better understanding of the hormonal regulation of BBM and BPM, it becomes important to establish the implication of G-proteins in Ca^{2+} transport during hormonal stimulation, since it is known that in some pathologies, the abnormal expression of the G-protein could alter the signal transduction pathways normally observed.

AC and PLC

There are only a few studies available regarding the identification and role of AC and protein kinase A (PKA) in the syncytiotrophoblast. The AC is an effector implicated in the cellular response following the stimulation of some receptors by their respective agonists, such as PTH, PTHrP, and calcitonin. Nine mammalian isoforms of this enzyme have been identified in a variety of tissues *(101)*. They are numbered AC I to AC IX and subdivided into separate groups according to their sensitivity to various stimulation agents. Group 1 (AC I, -III, -VIII) is stimulated by Ca^{2+} and calmodulin. Members of group 2 (AC II, -IV, -VII) are activated by the βγ-subunit and by PKC phosphorylation. Group 3 (AC V, -VI) is inhibited by a low concentration of Ca^{2+}, and group 4 (AC IX) is insensitive to either Ca^{2+} or the βγ-subunit *(101)*. They are all under the control of the α_s G-protein subunit and represent the initiating complex of the cAMP/PKA pathway *(77)*. In the placenta, AC is exclusively located on the BPM of the syncytiotrophoblast *(102)*, but currently there are no studies on the characterization of the different AC isoforms present in human syncytiotrophoblast.

PLC-β is recognized as the main regulator of diacylglyceride/PKC and inositol phosphates (IPs)/$[Ca^{2+}]_i$ pathways. Because PTH activates PLC in other organs involved in Ca^{2+} homeostasis, and calcitonin and PTHrP possess the potential to stimulate the formation of IPs in BBM and BPM of the human placental syncytiotrophoblast *(103, 104)*, we hypothesize that PLC could therefore be related to some mechanisms controlling Ca^{2+} transfer by the syncytiotrophoblast membranes. PLC has also been identified as the privileged pathway to activate PKC following stimulation by PTH and PTHrP in syncytiotrophoblast *(105)*.

Protein Kinases

PKA and PKC are mostly cytosolic proteins that will, following their activation, translocate toward the membrane in order to phosphorylate certain proteins containing serine and threonine residues. In turn, this will produce the biologic effects carried by the extracellular messenger *(106,107)* or will activate Ca^{2+} channels *(108)*. In the PKC family, many isotypes have been identified in various tissues and are classified in at least three subfamilies: the classic PKC (cPKC), the new PKC, and the atypical PKC. Only the cPKC isotypes are activated by the presence of Ca^{2+} *(109,110)*. Amane et al. *(111)* demonstrated, for the first time, the asymmetrical distribution of many isotypes from the three PKC subfamilies in BBM and BPM of the human syncytiotrophoblast, including the presence of the γ isotype, which has been previously identified only in innerved tissues *(109,112)* such as the brain. Therefore, it is not surprising to find this γ isotype in the syncytiotrophoblast since it possesses some characteristics of innerved tissues, such as the presence of β-adrenergic receptors on the BPM *(113)*. Because the activity of members of the PKC family is modulated by PTH and PTHrP *(105)*, it is possible that they could actively take part in the Ca^{2+} transfer by the syncytiotrophoblast, as is the case in the kidney *(114)*.

Tamanini et al. *(115)* have demonstrated the presence of the PKA catalytic subunit in human placenta. It has also been demonstrated that PKA inhibits the chloride channels located in the BBM of the human placenta, whereas it activates these channels in ox trachea *(116)*. Furthermore, Doolan and Keenan *(117)* have demonstrated that arachidonic, elaidic, and oleic acids have the capacity to inhibit the activity of the PKA located in the human placental vesiculated BBM.

In the other kinase class, the biologic responses of mitogen-activated protein kinase (MAPK) are associated with various signaling pathways. MAPKs consist of a family of serine/threonine kinases that includes the extracellular regulated kinases (ERKs , c-Jun, and p38) *(118,119)*. The basic structure of the MAPK cascade is well conserved. MAPK kinase kinase activate MAPK kinase (MAPKKs) by phosphorylation on two conserved serine residues. In turn, MAPKKs activate MAPKs by phosphorylation on two conserved threonine and tyrosine residues. The cascade relays signals from the plasma membrane to targets in the cytoplasm and the nucleus *(120)*. Robidoux et al. *(121)* have recently demonstrated the involvement of this signaling pathway in syncytiotrophoblast of human placenta following neuropeptide Y stimulation.

Signaling in Presence of Major Calcemic Hormones

Cell Signaling in Response to PTH and PTHrP

Until now, not much information has been available on signaling pathways in the syncytiotrophoblast in response to PTH and PTHrP, and even less on the link between these pathways and their involvement in Ca^{2+} flux. To modulate signaling pathways in the placenta, PTH and PTHrP must bind to their respective receptors. In fact, Lafond et al. *(122)* demonstrated the existence of the specific PTH receptors, similar to those found in renal and bone membranes, on both BBM and BPM of the human syncytiotrophoblast. However, their study did not distinguish among the different PTH and PTHrP receptor subtypes, such as PTH/PTHrP *(123)*, PTH2 *(124)*, and PTHrP *(125)* receptors. Usdin et al. *(126)* demonstrated the presence of mRNA of PTH2 receptor in the placenta, but they did not associate a specific function with this receptor. Urena et al. *(127)* identified mRNA of the PTH/PTHrP receptors in the rat placenta. Unfortunately, both of these studies did not verify the localization of the expressed protein.

Because all the receptors for these hormones belong to the superfamily of seven-transmembrane domains receptors coupled to various G-proteins, the PTH, PTHrP, and calcitonin receptors share a high degree of homology. Thereafter, their signals can modulate more than one effector. The most recent information demonstrates that cAMP from the AC pathway located on BPM regulates the transport of phosphate through the BBM, in response to PTH *(122,128)* by a mechanism that remains to be identified. Studies in other systems such as renal tubular cells *(129, 130)* and osteoblasts *(131)* showed that PTH increases inositol triphosphate (IP_3), inositol tetraphosphate, and diacylglyceride production, suggesting the resynthesis of phosphoinositides. In addition, other studies demonstrated the activation of two second messengers (cAMP and IP) in syncytiotrophoblast BPM and an increase in the IPs in BBM, following stimulation with PTH and PTHrP, respectively *(104,122)*. However, these two phenomena are independently accomplished and cannot be attributed to two different receptor subtypes *(132,133)*. Abou-Samra et al. *(134)* have shown an increase in cAMP and IP production, following the stimulation of rat osteoblast-like cells with PTH or PTHrP. These intracellular rises would be accomplished through the activation of α_q- and β-subunits of G-proteins in COS-1 cells *(135)*. In other tissues, such as the astrocytes, this affirmation does not apply since the presence of PTH activates AC but not PLC *(136)*.

PTH and PTHrP also require phosphorylation mechanisms (PKA and PKC) to transmit their cellular signals. It has been shown that PTH inhibits alkaline phosphatase in BBM, whereas PTHrP stimulates it, suggesting different roles for these two hormones in dephosphorylation mechanisms involving this enzyme in syncytiotrophoblast *(137)*. For example, Laramée et al. *(105)* demonstrated that PTHrP (fragment 1–40) stimulates the PKCs in BBM and BPM. Moreover, they demonstrated that MAPK could be involved in PTHrP signaling, since the inhibitor of phosphatidylinositol-3-kinase (PI3K) modulated the phosphorylation of PKC *(105)*. Thus, it is reasonable to believe that PTH and PTHrP could modulate transplacental Ca^{2+} transport using various cell transduction pathways.

Cell Signaling in Response to Calcitonin

There are only a few studies on signaling pathways activated by calcitonin. Stroop et al. *(138,139)* suggest that the physiologic state of the cellular and extracellular Ca^{2+} could be modified following the activation of the calcitonin receptor. In fact, Lafond et al. *(103)* showed the presence of a specific calcitonin receptor in syncytiotrophoblast BBM and BPM. This receptor, when stimulated with calcitonin, modulates the production of IPs in both membranes. Rizzo and Goltzman *(140)* demonstrated that calcitonin in human placental tissue activates the AC system, whereas it causes its inhibition in the rat hypothalamus. Moreover, Kuestner et al. *(141)* demonstrated that at a low concentration calcitonin increases the rate of cAMP production in the trophoblast, while a larger concentration increases the metabolism of the IPs and subsequently increases intracellular Ca^{2+}. This corroborates the results obtained by Force et al. *(133)* on the kidney. On the other hand, Rappaport and Stern *(142)* reported that calcitonin decreases phosphoinositol metabolism in bone and prevents the stimulating effect of PTH on the production of IP_3.

Table 1
Signaling Pathways Stimulated in Kidney, Bone, and Placental Models by PTH, PTHrP, and Calcitonin[a]

	Origin	Kidney	Bone	Placenta	Placental receptors
PTH	Parathyroid	↑ cAMP *(114)* ↑ IP_3 IP_4, DAG *(63–65,69)*	↑cAMP *(69)* ↑ ALP activity *(144)*	↑cAMP BPM *(122,127)* ↑ IP BBM *(104,122)* ↓ ALP activity BBM *(72)*	PTH2 *(60,62)* PTH/PTHrP *(59)*
PTHrP	Fetal parathyroid Placenta Tumors Other fetal tissus	↑ cAMP *(145)* ↑ PLC activity *(145)*	↑ cAMP *(146)* ↑ PLC activity *(147)*	↑ cAMP BPM *(104,122)* ↑ ALP activity BBM *(72)*	PTH/PTHrP BPM?
CT	Thyroid gland Placenta	low [CT] ↑ cAMP *(68)* high [CT] ↑ IP *(68)* ↑ ALP activity *(149)*	↑ cAMP *(148)* ↓ IP bone *(84)* ↑ PLC activity *(150)*	↑ IP BBM *(103)* ↑↑ IP BPM *(103)*	CT *(103)*

[a]PTH, parathyroid hormone; PTHrP, PTH-related peptide; CT, calcitonin; cAMP, cyclic adenosine monophosphate; IP_3, inositol triphosphate; IP_4, inositol tetraphosphate; DAG, diacylglyceride; PLC, phospholipase C; ALP, alkaline phosphatase; BPM, basal plasma membrane; BBM, brush-border membrane.

As for other signaling pathways, Chen et al. *(143)* demonstrated that the rabbit calcitonin receptor induces Shc tyrosine phosphorylation and Erk1/2 activation in a dual mechanism involving the α_i-subunit of the G-protein and PKC in stably transfected HEK 293 cells. All of these results show that calcitonin can also be involved in MAPK signaling. Table 1 summarizes a few signaling pathways stimulated by PTH, PTHrP, and calcitonin, in kidney, bone, and placenta models.

Calcium-Binding Proteins

Wasserman's group *(151–152)* discovered calbindins. They are intracellular components involved in the binding, shuttling, and regulation of the intracellular Ca^{2+}. As mentioned previously, in the human placenta, a few members of this family have been identified. The placental calbindins have two proposed roles: to serve as facilitator of Ca^{2+} diffusion from the BBM to the BPM and possibly to stimulate directly or indirectly the activity of the BPM Ca^{2+}-ATPase. The small 9-kDa calbindin (calbindin 9K) has two Ca^{2+} binding sites and the large 28-kDa one (calbindin 28K) has four to six binding sites. Synthesis of these calbindins in some organs is regulated by the active form of vitamin D, but in the uterus, the synthesis of calbindin 9K has been shown to be under the control of estrogen *(153)*. Thus, the genes encoding for rat placental 9-kDa CaBP *(154)* as well as the murine renal 28-kDa CaBP *(155)* have an estrogen-responsive element. Among other regulating factors, the mRNA, protein level of CaBP, and Ca^{2+} uptake are stimulated by the presence of PTHrP (fragment 67–84), whereas $1,25(OH)_2D_3$, PTH, PTHrP (fragment 1–34), and Ca^{2+} have no effect on these parameters. It is known that both calbindins are present in the placenta, but the majority of the studies on this topic were done in other tissues than human placenta *(156,157)*. Therefore, more studies are required to complete the identification and clarify the functions and regulations of such proteins in the placenta.

Cell Signaling Regarding Calcium Channels

Among the different types of existing channels, three types have been studied: nonvoltage-dependent calcium channels, receptor-operated Ca^{2+} channels, and especially VDCCs, which have recently been identified in the placenta *(158)*. There is controversy in the scientific literature about the presence of VDCCs in nonexcitable cells *(159)*, even though some studies have shown the presence of L-type Ca^{2+} channels in these cells *(160,161)*. In fact, Ca^{2+} could enter by cells channels as well as by carrier mechanisms.

Five classes of VDCC have clearly been defined *(162–164)*: L-type (long lasting), N-type (neuronal), P-type (Purkinje), T-type (transient), R-type (resting), and Q-type. The L-type, extensively studied, corresponds to the channel sensitive to 1,4-dihydropyridines *(165)*. The Ca^{2+} flux of this channel is regulated when it is phosphorylated by a cAMP-dependent mechanism and by the presence of the active α_s G-protein subunit *(166)*. However, in some systems this regulation is performed by different protein kinase systems, such as protein kinase G, PKC, and Ca^{2+}-calmodulin-protein kinase *(108,167)*. In this case, phosphatases, which dephosphorylate the channels, are important downregulators of the activity of these structures *(168)*. The majority of the different VDCC types can be classified by their specific antagonists. The L-type Ca^{2+} channels are blocked by antagonist drugs from the dihydropyridine class (e.g., nifedipine, nimodipine), phenylalkylamines (e.g., verapamil, D-600), and the diphenylpiperazines (e.g., flunarizine) and are opened by agonist drugs from the dihydropyridine class. These agonists, such as the BAY-K-8644 *(169)* and the PCA 50941 *(170)*, have undergone modification of their structure resulting in a structural homology with nifedipine. Some studies have demonstrated that the inhibitors of L-type Ca^{2+} channels have no influence on the basal Ca^{2+} transport by vesiculated BPM *(4)* and BBM *(13)*. Recently, Lafond et al. *(158)* have shown by immuno-

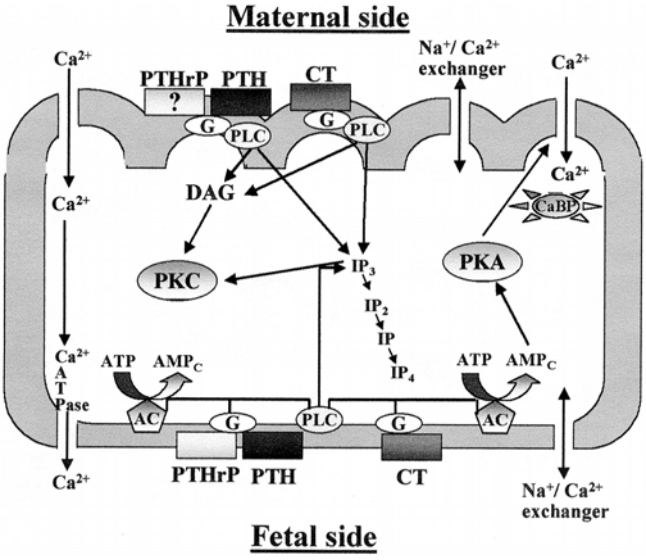

Fig. 2. Actual knowledge of the Ca^{2+} transport by the syncytiotrophoblast in human placenta. CT, calcitonin; DAG, diacylglyceride.

detection the presence of an L-type VDCC only in BBM from human syncytiotrophoblast. Regarding the other VDCC types, such as T-type *(171)*, N-type *(172)* or P-type *(173)*, they require a depolarization much too important to be present in the nonexcitable cells.

Conclusion

This overview of Ca^{2+} management during pregnancy allows us to appreciate the importance of dietary intake of this ion as well as the crucial hormonal balance for the maintenance of fetal and maternal health. PTH, PTHrP, and calcitonin are all potent hormones implicated in maintaining adequate Ca^{2+} levels of the mother and her relative hypercalcemic fetus. Because these hormones are agonists of G-protein-coupled receptors, they can be linked to the cAMP, DAG, and IPs as second messengers in order to activate PKA, PKC, or direct effectors involved in Ca^{2+} transfer from maternal to fetal circulation. The actual knowledge of the Ca^{2+} transport by the human syncytiotrophoblast is summarized in Fig. 2. Even though the literature on this topic is appreciable, more studies are needed to assess the site of action in the uteroplacental unit and signaling cascades of all the major calcemic hormones, as well as their involvement in the complex calcium homeostasis process.

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