Parathyroid hormone-related peptide (38–94) amide stimulates ATP-dependent calcium transport in the basal plasma membrane of the human syncytiotrophoblast

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Abstract

The final step in the maternal–fetal transfer of calcium in the placenta involves transport against a concentration gradient across the syncytiotrophoblast basal plasma membrane (BM). Based on animal studies, it has been proposed that parathyroid hormone-related peptide (PTHrP) plays a major role in maintaining the maternal–fetal concentration gradient of calcium. In this study, we tested the hypothesis that a highly conserved mid-region fragment (38–94) of PTHrP directly affects the ATP-dependent calcium transport across BM isolated from full-term human placentas. PTHrP (38–94) stimulated ATP-dependent calcium transport at a concentration within the physiological range (5 pg/ml) and the effect (10–38% increase) was concentration dependent over the range 5 pg/ml to 5 ng/ml

($n=8$; $P<0.05$). In contrast, PTH, PTHrP (1–34), PTHrP (67–86) and calcitonin increased BM calcium transport only at concentrations much higher than physiological. The increased calcium uptake was inhibited by the protein kinase C (PKC) inhibitor chelerythrine ($n=6$; $P<0.05$). In addition, PTHrP (38–94) increased inositol trisphosphate (IP_3) production and PKC phosphorylation in human placental BM ($n=12$; $P<0.05$). Our data indicate that PTHrP (38–94) stimulates Ca^{2+} ATPase in the human syncytiotrophoblast BM vesicles by activating the IP_3 –DAG–PKC pathway. We suggest that PTHrP (38–94) is important in maintaining the calcium concentration gradient across the placental barrier in the human.

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Introduction

Parathyroid hormone-related peptide (PTHrP) was originally discovered in tumour cells through its structural and functional homology with parathyroid hormone (PTH) and was found to interact with PTH 1 receptors in bone and kidney to cause hypercalcaemia of malignancy (Stewart *et al.* 1980, Strewler *et al.* 1987). Subsequently, it has been demonstrated that PTHrP plays an important physiological role and is involved in embryonic development, cellular differentiation and proliferation, regulation of smooth muscle contraction and in transepithelial transport (Philbrick *et al.* 1996). PTHrP is produced in various tissues and may act in autocrine, paracrine and endocrine fashions. The molecule is produced as a 141 amino acid long pro-hormone which is cleaved into different active fragments with distinct functions. In addition to PTHrP (1–141), the human gene encodes (1–173) and (1–139) species (Wysolmerski & Stewart 1998). The most studied segment of the pro-hormone is PTHrP (1–34), which shows considerable sequence homology to PTH and activates the common PTH/PTHrP receptor (Gardella

et al. 1995). In contrast, the mid-molecule segment of PTHrP has no homology to PTH and has been shown to interact with a unique receptor; however, it has not yet been identified (Kovacs *et al.* 1996).

The expression of the PTHrP mid-molecule in reproductive tissue has not been studied, whereas mRNA expression of PTHrP pro-hormone as well as protein expression of PTHrP (1–34) and PTHrP (1–86) have been investigated in some detail. During normal human pregnancy, PTHrP is produced in the placenta, umbilical cord, amnion, chorion, decidua and myometrium (Ferguson *et al.* 1992, Germain *et al.* 1992, Bowden *et al.* 1994, Bruns *et al.* 1995, Curtis *et al.* 1997) as well as in fetal parathyroid glands, bone, skin, gut and lung (Moniz *et al.* 1990, Williams *et al.* 1994). PTHrP receptor mRNA has been identified in most of these tissues, compatible with autocrine and paracrine actions of PTHrP (Ferguson *et al.* 1992, Germain *et al.* 1992, Wlodek *et al.* 1995). Furthermore, PTHrP expression is more abundant in the amnion than in other intrauterine tissues and the high PTHrP concentrations in amniotic fluid may affect the placenta and fetus in a paracrine fashion (Wlodek *et al.* 1995).

Indeed, PTHrP levels in human amniotic fluid increase in late pregnancy coinciding with rapid fetal growth and calcium accretion in the third trimester (Curtis *et al.* 1997). In addition, PTHrP can be detected in fetal blood in concentrations higher than those in the maternal circulation, suggesting an endocrine function of the hormone in the fetus (Seki *et al.* 1994, Bucht *et al.* 1995). In mice with homozygous deletion of the PTHrP gene, placental transport of calcium is decreased and the normal concentration gradient for calcium across the placental barrier fails to develop. In these studies, transplacental calcium transport was increased in homozygous fetuses by administration of PTHrP (1–86) or PTHrP (67–86), but not by PTHrP (1–34) or intact PTH (Kovacs *et al.* 1996). In addition, Care *et al.* (1990) have previously shown that a synthetic PTHrP mid-molecule fragment (67–86) maintains the calcium gradient across the placenta in sheep. This work was later extended by Wu *et al.* (1996), who demonstrated a similar effect with the highly conserved mid-region secretory fragment of PTHrP (PTHrP (38–94) amide). However, none of these studies addressed the mechanism of action of the mid-region of PTHrP. In studies in the rat, PTHrP (1–34) and PTHrP (75–86) had no significant effect on transplacental calcium transport (Shaw *et al.* 1991). Likewise, the findings in the sheep could not be reproduced in the pig following removal of the fetal parathyroid glands (Care *et al.* 1990, Abbas *et al.* 1994). These experimental studies suggest that the mid-molecular region of PTHrP might play a critical role in some species in stimulating placental transport of calcium and raises the possibility that PTHrP mid molecule may have a similar function in the human.

The final step in maternal–fetal transfer of calcium in the human involves transport against a steep concentration gradient across the basal membrane (BM), the fetal-facing plasma membrane of the syncytiotrophoblast. BM vesicles exhibit a high ATP-dependent Ca^{2+} transport activity, which, to a larger extent, can be attributed to plasma membrane Ca^{2+} ATPase (PMCA) (Fisher *et al.* 1987, Lafond *et al.* 1991, Strid & Powell 2000). Two isoforms of PMCA have been identified at the protein level in BM and in the syncytiotrophoblast cell at the mRNA level (Howard *et al.* 1992, Strid & Powell 2000). In late gestation, calcium transport across the human placenta must increase in response to the demands of the accelerating bone mineralization of the fetus (Shaw 1976, Ziegler *et al.* 1976, Minton *et al.* 1983, Ryan *et al.* 1988). We have demonstrated previously that the ATP-dependent calcium uptake into BM vesicles increased linearly during the third trimester, whereas PMCA protein expression remained unaltered (Strid & Powell 2000). However, the factor that might stimulate the activity of BM PMCA in late gestation is unknown. In this study, we test the hypothesis that PTHrP (38–94) amide stimulates the ATP-dependent calcium transport into BM isolated from term human placentas.

Materials and Methods

Materials

General chemicals and PTH were bought from Sigma-Aldrich, Stockholm, Sweden. The protein kinase A (PKA) inhibitor amide 5–24 and chelerythrine chloride were obtained from Calbiochem, Darmstadt, Germany. An inositol trisphosphate (IP_3) [^3H] assay system and $^{45}\text{CaCl}_2$ were purchased from Amersham Pharmacia Biotech AB, Uppsala, Sweden. Synthetic PTHrP (38–94) amide and PTHrP (37–74) were generous gifts from Professor A F Stewart, University of Pittsburgh. PTHrP (38–94) amide used throughout this study will be referred to as PTHrP (38–94). The source of human PTHrP (1–34) and human calcitonin (hCT) was Bachem Ltd, Saffron Walden, Essex, UK.

Vesicle preparation

Human placentas were obtained from uncomplicated term pregnancies. Tissue was processed within 30 min of vaginal or Caesarean delivery. The collection of placental tissue was approved by the Committee for Research Ethics at Göteborg University. The basal (BM) and the microvillous membrane (MVM) of the syncytiotrophoblast were isolated simultaneously by the method of Illsley *et al.* (1990) with some modifications. Briefly, the chorionic plate and decidua were removed and the tissue was cut into small pieces. The tissue was homogenized in buffer D (250 mmol/l sucrose, 10 mmol/l Hepes/Tris, 1.6 $\mu\text{mol/l}$ antipain, 0.7 $\mu\text{mol/l}$ pepstatin A, 0.5 $\mu\text{mol/l}$ aprotinin and 1 mmol/l EDTA) followed by centrifugation of the homogenate at 10 000 *g* at 4 °C for 15 min. The separation of the two membranes was performed by adding magnesium chloride (12 mmol/l) to the supernatant, which was stirred on ice for 20 min. The mixture was then centrifuged at 2500 *g* for 10 min at 4 °C. The pellet contained the BM and the supernatant MVM. The BM was further purified by sucrose gradient centrifugation (Illsley *et al.* 1990). BM vesicles were snap frozen in liquid nitrogen and stored at –80 °C.

Characterization of BM preparation

Membrane enrichment was assessed using standard activity assays for adenylate cyclase (Schultz 1984) and alkaline phosphatase (Bowers & McComb 1966). The production of cAMP by adenylate cyclase was measured by radioimmunoassay (RIA) (New England Nuclear, Boston, MA, USA). The enrichment values were calculated as the ratio of the enzyme activity in the purified membrane preparation and the activity in the homogenate (alkaline phosphatase) or post-nuclear membrane fraction (adenylate cyclase). The results are presented in Table 1. The enrichment in adenylate cyclase (BM marker) activity was

Table 1 Basal plasma membrane enzyme activities and enrichments. Enrichments values were calculated as the ratio of activity in the purified membrane compared with the activity in homogenate (Hom)* or post-nuclear membrane fraction (P2)**. Values are means $\pm$ S.E.M., $n=6$

Assay	BM	Hom/P2	Enrichment
Alkaline phosphatase (nmol PO ₄ /s per mg protein)	6.9 $\pm$ 1.3	2.4 $\pm$ 0.8	2.9*
Adenylate cyclase (pmol/min per mg protein)	514 $\pm$ 67	26 $\pm$ 8	19.8**

19.8-fold while alkaline phosphatase (MVM marker) activity was 2.9-fold in our BM preparation. We have previously shown that the degree of contamination by endoplasmic reticulum and mitochondria is low in our BM preparation (Strid & Powell 2000).

Calcium uptake

The uptake of $^{45}\text{Ca}^{2+}$ by BM vesicles was measured by standard rapid filtration techniques (Fisher *et al.* 1987) and the calcium uptake was measured at an initial rate after 10 min of incubation (Strid & Powell 2000). The vesicles were resuspended in vesicle buffer (10 mmol/l Hepes/Tris and 250 mmol/l sucrose, pH 7.4 at 37 °C) and centrifuged at 50 000 g at 4 °C for 30 min. After centrifugation, the vesicles were resuspended in vesicle buffer containing 0.1% bovine serum albumin (BSA) in the presence or absence of 5 mmol/l ATP–MgCl₂. BM vesicles were loaded with effectors (PTHrP (38–94), PTHrP (67–86), PTHrP (1–34), PTHrP (37–74), PTH, hCT or forskolin) by a gentle freeze–thaw technique (Donowitz *et al.* 1987). Briefly, the effector was added to the vesicle suspension at desired concentrations and mixed. The tubes were then placed in liquid nitrogen for 5 min and subsequently on ice for 90–120 min. After the freeze–thaw procedure, the tubes were preincubated for 30 min in a 37 °C water bath. Subsequently, calcium uptake measurements were started by the addition of 20 μ l vesicles (100–200 μ g total membrane protein) into 500 μ l calcium transport buffer (240 mmol/l sucrose, 5 mmol/l MgCl₂, 0.2 mmol/l EGTA, 0.2 mmol/l CaCl₂, 1 μ Ci $^{45}\text{CaCl}_2$ and 10 mmol/l Hepes/Tris, pH 7.4) in the presence or absence of 5 mmol/l ATP–MgCl₂. Effectors in the appropriate concentration were also added to the reaction mixture. Calcium uptake was allowed to proceed for 10 min at 37 °C and stopped by the addition of an ice-cold wash solution (10 mmol/l Hepes/Tris, 250 mmol/l sucrose and 4 mmol/l EGTA, pH 7.4 at 4 °C). Subsequently, the reaction mixture was filtered under vacuum on 0.45 μ m filters (HAWPO2500; Millipore, Bedford, MA, USA) that had been prewashed with 10 mmol/l Hepes/Tris, 250 mmol/l sucrose and 5 mmol/l CaCl₂ (pH 7.4 at 4 °C). The filter was washed three times with 2 ml 10 mmol/l Hepes/Tris and 250 mmol/l sucrose (pH 7.4 at 4 °C). The radioactivity retained on the filter was measured by liquid scintillation. The ATP-dependent calcium uptake was

calculated as the difference between Ca^{2+} uptake in the presence and the absence of ATP. Calcium uptake is expressed as nmol Ca^{2+} /mg total membrane protein. In most experiments, the calcium uptake in the presence of effectors is given as a relative value in relation to controls, which are assigned an arbitrary value of one.

In some experiments, chelerythrine, an inhibitor of protein kinase C (PKC) or the PKA inhibitor amide 5–24 was added to the vesicle mixture just prior to the 30 min preincubation. Subsequently, calcium uptake was measured as described above.

cAMP measurement

BM vesicles (100–200 μ g) were incubated in 500 μ l calcium transport buffer containing 5 mmol/l ATP–MgCl₂ and in the presence or absence of effectors (PTH, 250 ng/ml; hCT, 1 ng/ml; PTHrP (1–34), 250 ng/ml; PTHrP (38–94), 0.5 ng/ml; forskolin, 30 μ M). Experiments carried out in the absence of ATP–MgCl₂ served as blanks. After 10 min of incubation, 400 μ l ZnCl₂ and 500 μ l Na₂CO₃ were added to 100 μ l reaction solution. The tubes were then centrifuged at 10 000 g for 5 min at room temperature. cAMP was measured by RIA (New England Nuclear, Boston, MA, USA).

IP₃ measurement

The effect of PTHrP (38–94) on IP₃ production was measured in BM from full-term placentas by an assay system using D-myo [³H]inositol 1,4,5-trisphosphate as a tracer (Amersham Pharmacia Biotech AB, Uppsala, Sweden). BMs were subjected to a freeze–thaw procedure as described above (Donowitz *et al.* 1987) in the presence or absence of 500 pg/ml PTHrP (38–94) and in the presence of 5 mmol/l ATP–MgCl₂. The reaction was stopped by placing samples on ice and 100 μ l aliquots of the sample were added to the assay buffer. Thereafter, the assay was performed according to the protocol provided by the manufacturer. The samples were counted in a liquid scintillation counter and the results were calculated from a standard curve performed at the same time as the experiment.

PKC phosphorylation

Phosphorylation of PKC was measured in BM from full-term placentas in the presence or absence of PTHrP

(38–94) (500 pg/ml), by using a PKC enzyme assay system (Amersham Pharmacia Biotech AB). BM vesicles were subjected to a freeze–thaw procedure (Donowitz *et al.* 1987). Subsequently, 25 µl aliquots of the BM sample (75–150 µg protein) were added to the assay buffer followed by the addition of [32 P]ATP buffer according to the protocol provided by the manufacturer. The samples were incubated in a 37 °C water bath for 15 min and the reaction was ended by a stop buffer provided in the assay system. The samples were counted in a liquid scintillation counter and the results were calculated from the specific activity of 1.2 mM Mg [32 P]ATP.

Protein determination

All protein concentrations were determined according to the method of Bradford (Bradford 1976) using the Bio-Rad protein assay procedure (Bio-Rad, Hercules, CA, USA) and BSA as a standard.

Data analysis

Data are expressed as means $\pm$ S.E.M. unless otherwise specified. Statistical significance of differences between groups was determined by paired *t*-test or repeated measurement ANOVA and Dunnett's test.

Results

ATP-dependent Ca^{2+} transport

Calcium transport into BM in the absence of Mg–ATP was 0.6 ± 0.06 nmol Ca^{2+} /mg total membrane protein in 10 min, whereas the ATP-dependent transport of calcium was 21.2 ± 1.4 nmol Ca^{2+} /mg total membrane protein in 10 min ($n=8$). PTHrP (38–94) did not affect BM calcium transport in the absence of ATP (results not shown).

The ATP-dependent transport of calcium was increased (10–38%) with increasing concentrations of PTHrP (38–94) (5 pg/ml–5 ng/ml). Concentrations of hormone higher than 5 ng/ml did not stimulate ATP-dependent calcium transport further (Fig. 1; $n=8$; $P<0.05$; ANOVA). ATP-dependent calcium transport was unaltered by incubation with PTHrP (37–74) (250 ng/ml). We studied the effect of PTH, PTHrP (1–34) and PTHrP (67–86) on calcium transport in BM. These three hormones significantly increased the ATP-dependent transport but only at very high concentrations of PTHrP (1–34) (+19%; 50 ng/ml; $n=8$; $P<0.05$; Fig. 2A), PTH (+19%; 250 ng/ml; $n=8$; $P<0.05$; Fig. 2B) and PTHrP (67–84) (43%; 250 ng/ml; $n=6$; $P<0.05$). Similarly, both hCT (+32% at 5 ng/ml; $n=6$; $P<0.05$) and forskolin (+14%; 30 µmol/l, $n=4$; $P<0.05$) increased ATP-dependent calcium transport.

Figure 1 Stimulation of ATP-dependent calcium transport into BM as a function of PTHrP (38–94) concentration. ATP-dependent Ca^{2+} transport in the absence of hormone (control) was 21.2 ± 1.4 nmol/mg protein in 10 min. Uptakes in the presence of hormone are given as relative values in comparison with control, which was assigned a value of 1. Error bars for uptakes at 5 and 50 pg/ml fall within the symbol ($n=8$). * $P<0.05$ compared with control (ANOVA).

Effect of PTHrP on cAMP production

Using the hormone concentrations shown to increase ATP-dependent calcium uptake, PTH, PTHrP (1–34) and PTHrP (38–94) stimulated cAMP production by approximately 80% (Fig. 3). Furthermore, forskolin (30 µmol/l) increased cAMP production by fivefold (Fig. 3), the same concentration as that which stimulated the ATP-dependent calcium transport by only 14%. hCT (5 ng/ml) increased the cAMP production by 54%. All increases in cAMP production were significant compared with control experiments in which no effector was added ($n=7$; $P<0.05$; Fig. 3).

Effect of PKA and PKC inhibitors on ATP-dependent calcium transport

ATP-dependent calcium transport was not altered by the PKA inhibitor 5–24 (1 µmol/l–250 µmol/l; data not shown) whereas the presence of the PKC inhibitor, chelerythrine (1 µmol/l–250 µmol/l), significantly reduced ATP-dependent calcium transport in a concentration-dependent manner (Fig. 4). In addition, 50 µmol/l chelerythrine reduced total ATP-dependent

Figure 2 Effect of (A) PTHrP (1–34) and (B) PTH on the ATP-dependent calcium transport into BM. Results are expressed as relative values in comparison with control ($n=8$). * $P<0.05$ compared with control (ANOVA).

Figure 3 The effect of various effectors on cAMP production in isolated BM in the presence of 5 mmol/l ATP-MgCl₂. In control experiments no effector was added. Error bars for control uptake fall within the symbol ($n=7$). * $P<0.05$ compared with control (ANOVA).

Figure 4 The effect of the PKC inhibitor, chelerythrine, on ATP-dependent Ca²⁺ uptake into BM vesicles, relative to control. For uptakes at chelerythrine concentrations ≥ 10 μ M, the error bars fall within the symbol ($n=6$). * $P<0.05$ compared with control (ANOVA).

calcium transport in BM by 84%. The addition of 500 pg/ml PTHrP (38–94) did not result in increased calcium transport in the presence of chelerythrine ($n=6$; $P<0.05$; Fig. 5).

Effect of PTHrP on IP₃ production and PKC phosphorylation

The PKC inhibition study suggested that PTHrP (38–94) might activate the ATP-dependent calcium transport

through the IP₃–diacylglycerol(DAG)–PKC pathway in BM vesicles prepared from full-term placentas. We therefore incubated BM with PTHrP (38–94) and measured IP₃ production. At 500 pg/ml, PTHrP (38–94) stimulated IP₃ production by 33% ($n=12$; $P<0.05$; Fig. 6A). Furthermore, PTHrP (38–94) (500 pg/ml) also increased PKC phosphorylation by 25% in BM ($n=12$; $P<0.05$; Fig. 6B).

Figure 5 The effect of the PKC inhibitor, chelerythrine (50 µM), on basal and PTHrP (38–94)-stimulated ATP-dependent Ca^{2+} transport into BM. Error bars for uptakes in the presence of PTHrP (38–94) fall within the symbol ($n=6$). * $P<0.05$ compared with control (ANOVA).

Discussion

The main finding of this study is that ATP-dependent calcium transport by human syncytiotrophoblast BM vesicles was stimulated by PTHrP (38–94). Furthermore, we have provided evidence that PTHrP (38–94) activates the Ca^{2+} -ATPase in the human syncytiotrophoblast BM through the IP_3 –DAG–PKC second messenger pathway.

All the effectors used in this study stimulated the ATP-dependent calcium transport in BM. However, only PTHrP (38–94) increased calcium transport at a concentration that was within the physiological range. PTHrP

(38–94) significantly stimulated PMCA activity at 5 pg/ml, which is similar to the concentration in umbilical cord blood (Bucht *et al.* 1995, Hirota *et al.* 1997). In addition, since PTHrP is synthesized locally, e.g. in the placenta (Deftos *et al.* 1994), PTHrP may have autocrine/paracrine effects and the hormone concentrations in the vicinity of the placental barrier may be substantially higher than indicated by the levels found in the fetal circulation. In contrast, PTH modestly stimulated ATP-dependent calcium transport in BM at a concentration of 250 ng/ml, which is 1000-fold higher than the concentrations found in the umbilical circulation (Saxe *et al.* 1997). Similarly, PTHrP (1–34) and calcitonin affected calcium transport activity in BM at concentrations that are higher than physiological (Pitkin *et al.* 1980, Hirota *et al.* 1997). Furthermore, PTHrP (67–86), one of the most studied mid-molecular fragments of PTHrP, did not affect PMCA activity at physiological concentrations. Our finding is consistent with earlier studies by Wu *et al.* (1996) demonstrating that PTHrP (38–94) is the fragment of PTHrP that plays a role in calcium transport across the sheep placenta. Furthermore, PTHrP (37–74) failed to stimulate calcium transport across the sheep placenta *in vivo* (Wu *et al.* 1996) and did not affect BM PMCA activity in our *in vitro* system. Indeed, the lack of effect of PTHrP (37–74) strongly suggests that the observed stimulatory effect of PTHrP (38–94) on BM PMCA is specific and indicates that the (75–94) region of PTHrP is important in eliciting the response.

In order to identify possible cellular signalling mechanisms mediating the stimulatory effect of PTHrP (38–94) on BM calcium transport, we studied the effect of inhibition of PKA and PKC. Whereas PKA inhibition did not affect basal or PTHrP (38–94)-stimulated PMCA activity, inhibition of PKC markedly reduced the basal ATP-dependent Ca^{2+} uptake in BM vesicles, suggesting that PMCA in BM *in vitro* is basally stimulated by PKC. In addition, PKC inhibition abolished the stimulatory effect

Figure 6 Production of (A) IP_3 and (B) phosphorylation of PKC in placental BM in the absence and presence of PTHrP (38–94) (500 pg/ml; $n=12$). P, phosphate. * $P<0.05$ compared with control (paired t -test).

of PTHrP (38–94) on ATP-dependent calcium uptake. These data suggest that BM PMCA is not maximally activated by PKC under basal conditions in term placenta and that PKC is involved in the second messenger pathway mediating the stimulating effect of PTHrP (38–94) on PMCA activity. Our data demonstrating increased production of IP₃ in placental BM as well as PKC phosphorylation in response to PTHrP (38–94) give additional support to the conclusion that binding of PTHrP mid-molecule to receptors in human placenta activates the IP₃–DAG–PKC pathway. These findings raise the possibility that PTHrP (38–94) may stimulate calcium transport *in vivo* also by IP₃-mediated mobilization of calcium stores in endoplasmic reticulum.

Production of cAMP is involved in the signalling following hormone binding to receptors for PTH, CT and PTHrP (1–34) (Rebut-Bonneton *et al.* 1992, Philbrick *et al.* 1996). In order to assess whether cAMP production could account for the stimulatory effect of PTHrP (38–94) on BM calcium transport, we measured cAMP production in BM in response to forskolin and PTHrP (38–94). Forskolin (30 µmol/l) increased cAMP production in BM vesicles fivefold, whereas PTHrP (38–94) (500 pg/ml) enhanced cAMP production by 80%. Using the same concentrations of these effectors we found that PTHrP (38–94) was twice as effective as forskolin in stimulating ATP-dependent calcium transport. These findings, together with the lack of effect of PKA inhibition on ATP-dependent calcium transport suggest that the stimulatory effect of PTHrP (38–94) on PMCA activity is not primarily mediated by cAMP. Consistent with this conclusion, Wu *et al.* (1996) showed, in three different cell lines, that PTHrP (38–94) did not significantly stimulate adenylate cyclase but did induce a brisk cytosolic calcium transient. Furthermore, the increased cAMP production after incubation with PTHrP (38–94) may be due to PKC activation since ‘cross-talk’ between these second messenger systems is well established (Jacobowitz *et al.* 1993, Yoshimura & Cooper 1993).

Recently, Farrugia *et al.* (2000) reported effects of PTH, PTHrP (1–34) and PTHrP (67–94) on ATP-independent calcium transport in human syncytiotrophoblast MVM and BM. PTH (1–34) (200 ng/ml) and PTHrP (1–34) (50 ng/ml) increased ATP-independent calcium transport in both plasma membranes, whereas PTHrP (67–94) had no effect. *In vivo*, calcium is transported across BM against a formidable concentration gradient, between the syncytiotrophoblast and the fetal circulation, and is, by definition, an active (ATP-dependent) transport process, primarily mediated by PMCA. Consequently, the implications of the findings of Farrugia *et al.* (2000) for calcium transport across BM in the fetal direction remain unclear.

In sheep and mice, it has been shown that PTHrP (38–94), but not PTH or PTHrP (1–34), is necessary for the establishment and maintenance of the concentration gradient for calcium across the placental barrier (Rodda

et al. 1988, Kovacs *et al.* 1996). In the present study, we have provided evidence to suggest that PTHrP (38–94) may play a similar role in human pregnancy. Such a role is indirectly supported by the findings that PTHrP mRNA expression in amnion and PTHrP concentrations in amniotic fluid increase in late pregnancy (Ferguson *et al.* 1992, Wlodek *et al.* 1995), a period during which fetal demands for calcium (Minton *et al.* 1983, Ryan *et al.* 1988) as well as BM PMCA activity increase rapidly (Strid & Powell 2000).

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