Parathyroid hormone(1–34) and parathyroid hormone-related protein(1–34) stimulate calcium release from human syncytiotrophoblast basal membranes via a common receptor

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Abstract

The placental syncytiotrophoblast is the site for mineral and nutrient exchange across the maternal–fetal interface. It has been proposed that parathyroid hormone-related protein (PTHrP) is a key factor in the maintenance of a maternal–fetal calcium gradient. Using simultaneously prepared microvillous (maternal facing) and basal (fetal facing) syncytiotrophoblast membranes from term human placentae ($n=8$), we determined the relative contribution of PTH(1–34), PTHrP(1–34) and PTHrP(67–94) to the regulation of syncytiotrophoblast calcium efflux. The vesicles had correct right-side-out membrane orientation and specific markers validated the fractionation of microvillous and basal membrane vesicles. Calcium efflux was studied by preloading vesicles with calcium-45 in the presence of calcium and magnesium and then incubating the vesicles at 37 °C for 15 min with the peptides. In basal membranes, PTHrP(1–34) significantly stimulated calcium efflux at a dose of 12·5 nmol/l, whereas PTH(1–34)-stimulated efflux was significant at 50 nmol/l ($P<0·05$, ANOVA). This efflux was significantly reduced in the presence of the PTH/PTHrP receptor antagonist (PTHrP(7–34)). Midmolecule PTHrP(67–94) had no significant effect on basal membrane calcium efflux. PTH(1–34), PTHrP(1–34) or PTHrP(67–94) had no significant effects on MVM calcium efflux. This study, using the human syncytiotrophoblast *in vitro* membrane system, demonstrated that PTHrP(1–34) and PTH(1–34) stimulate calcium transport across the basal, but not microvillous, syncytiotrophoblast membrane vesicles, mediated via the PTH/PTHrP receptor.

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Introduction

Parathyroid hormone-related protein (PTHrP) is produced by many tissues, in which it is believed to have a number of physiological roles, particularly in the mother during pregnancy and in the developing fetus (Philbrick *et al.* 1996, Wysolmerski & Stewart 1998). PTHrP, PTHrP mRNA and the common PTH/PTHrP receptor have been identified in gestational tissues, including, myometrium, fetal membranes and placenta (Ferguson *et al.* 1992, Germain *et al.* 1992, Bowden *et al.* 1994, Curtis *et al.* 1997, 1998, Thiede *et al.* 1990), and in fetal tissues (Moniz *et al.* 1990, Moseley *et al.* 1991, Dunne *et al.* 1994). In the placenta, PTHrP and its mRNA have been identified in both syncytiotrophoblast and cytotrophoblast cells (Deftos *et al.* 1994, Dunne *et al.* 1994). Potential autocrine, paracrine and endocrine roles during pregnancy that have been proposed for PTHrP include regulation of cellular growth and differentiation (Alsat *et al.* 1993, Lee *et al.* 1995), vasodilatation of the uteroplacental vasculature (Mandsager *et al.* 1994, Macgill *et al.* 1997), relaxation of uterine muscle (Thiede *et al.* 1990, Williams *et al.* 1994, Dalle *et al.* 1992, Paspaliaris *et al.* 1992) and stimulation of placental calcium transport (Rodda *et al.* 1988, Care *et al.* 1990, Kovacs *et al.* 1996).

The placenta is responsible for the transfer of nutrients from the mother to the fetus. The fetus is normally hypercalcaemic with respect to its mother, and effective placental calcium transfer between the mother and the fetus requires an active transport process across the syncytiotrophoblast (Sideri *et al.* 1983). Although little is known regarding the regulation of human placental calcium transport, recent evidence from sheep suggests that PTHrP may be an important modulator of placental

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calcium transport. PTHrP derived from the fetal parathyroids is critical for the maintenance of the active placental calcium gradient, as demonstrated in thyroparathyroidectomy experiments in fetal lambs (Care *et al.* 1990, Rodda *et al.* 1988) and decapitation studies on fetal rats (Robinson *et al.* 1989). Using placental perfusion in the thyroparathyroidectomized fetal sheep, it has been shown that partially purified fetal parathyroid extracts, PTHrP(1–108), PTHrP(1–141) and PTHrP(67–86) amide, but not PTH(1–34), PTH(1–84) or PTHrP(1–34), increased placental calcium transport (Rodda *et al.* 1988, Care *et al.* 1990). In contrast, the findings of other sheep studies demonstrated an increase in placental calcium transport in response to PTHrP(1–34) and PTH(1–34) (Barlet *et al.* 1990). However, in these animals the presence of intact parathyroids, kidneys and skeleton complicate the interpretation of the data (Barlet *et al.* 1990). In another model in which fetal rats had been cervically dislocated to effect parathyroidectomy, PTH(1–34) failed to have an effect on placental calcium transport (Robinson *et al.* 1989, Shaw *et al.* 1991). More recently, the work of Kovacs and colleagues (1996) has supported the notion that placental calcium transport is regulated by a midmolecule fragment of PTHrP. They showed that homozygous *PTHrP* gene knockout fetal mice of heterozygous mothers have reduced placental calcium transport (Kovacs *et al.* 1996, Tucci *et al.* 1996), which is increased by treatment with PTHrP(1–86) or PTHrP(67–86), but not PTHrP(1–34) or PTH peptides (Kovacs *et al.* 1996). Although *PTH/PTHrP* receptor gene knockout mice are hypocalcaemic, the observation that placental calcium transport was increased indicates that the PTH/PTHrP receptor was not required for this function (Kovacs *et al.* 1996). Early evidence in the sheep model, including the identification of PTHrP within the placenta, suggested that, early in gestation, PTHrP derived from placenta is important in the maintenance of the maternal–fetal calcium gradient and this role may be taken over, at least in part, after further development of the fetal parathyroids later in gestation (Rodda *et al.* 1988, MacIsaac *et al.* 1991). It is possible that there are species differences in placental calcium regulation, but this remains to be established.

Transfer of calcium occurs across two structurally and functionally distinct regions of the placental syncytiotrophoblast's plasma membrane, the maternal-facing microvillous membrane (MVM) and a fetal-facing basal membrane (BM) (Sideri *et al.* 1983). Calcium is believed to enter the syncytiotrophoblast at the MVM passively down a concentration gradient. Entry of calcium occurs through channels or through calcium-binding proteins at the membrane surface. Calcium is moved across the cell by calcium-binding proteins that also act to buffer the changing intracellular concentrations and therefore assist in the maintenance of the concentration gradient. At the BM, calcium is extruded from the cell actively by a Ca^{2+}–ATPase exchange mechanism (Fisher *et al.* 1987, Borke *et al.* 1989). The active extrusion of calcium at the BM is suggested to be the rate-limiting step of placental calcium transport. An *in vitro* differential centrifugation and magnesium precipitation technique has been used in the isolation and purification of these two distinct membrane regions of the human syncytiotrophoblast (Smith *et al.* 1974, Kelley *et al.* 1983, Lafond *et al.* 1988, Illsley *et al.* 1990), and this technique provides a useful model with which to study placental calcium transport without the confounding effects of intact fetal parathyroids, skeletal or renal demands. This study aimed to investigate the role of PTHrP and the PTH/PTHrP receptor in the regulation of calcium transport across the human syncytiotrophoblast, using MVM and BM vesicles prepared by differential centrifugation and magnesium precipitation.

Materials and Methods

Reagents

All reagents were obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise stated.

Tissue collection

Human placentae were obtained (with Royal Women's Hospital, Research and Ethics Committee approval) at elective caesarean section before the onset of labour from uncomplicated pregnancies. Tissues were received in the laboratory within 10–15 min of delivery.

Preparation of vesicles

Vesicle preparation was according to the Illsley method, with a number of modifications (Illsley *et al.* 1990). All procedures were performed at 4 °C unless otherwise stated. Duplicate 40 g samples of fresh placental tissue were homogenized in 80 ml Buffer 1 (250 mmol/l sucrose, 10 mmol/l Hepes, pH 7·0 containing 1 nmol/l protease inhibitor 4-(2-aminoethyl)-benzenesulphonyl fluoride) for 20 s, using a metal-blade tissue homogenizer (Ultra-turrax, T25 and S25N 8 G dispersing tool; Jenke and Kunkle GMBH and Co., Staufen, Germany). The homogenate was centrifuged at 10 000 ***g*** for 10 min (Beckman JA14) and the supernatant collected. The pellet was re-extracted in Buffer 1 and recentrifuged at 10 000 ***g*** for a further 10 min. The pooled supernatants were then centrifuged at 40 000 ***g*** for 60 min. The resultant pellet was resuspended in 12 ml Buffer 1 and homogenized with ten strokes of a Dounce glass homogenizer. MVMs were prepared by adding 4 mol/l $MgCl_2$ (final concentration of 12 mmol/l) and rocked on ice for 20 min. This solution was then centrifuged at 2500 ***g*** for 15 min to sediment the Mg^{2+}-aggregated MVM, which was resuspended in 2 ml Buffer 2 (250 mmol/l sucrose, 10 mmol/l Hepes, pH 7·0) in a Dounce homogenizer. MVMs were aliquoted, frozen in liquid N_2 and stored at − 80 °C. The supernatant was

further centrifuged at 40 000 ***g*** for 30 min. The BM pellet was resuspended in 2 ml Buffer 2 with a Dounce homogenizer and applied to a continuous 20% Percoll sucrose gradient (preformed by centrifugation at 30 000 ***g*** for 30 min). The gradient was centrifuged at 40 000 ***g*** for 45 min using a Beckman JA 20 fixed rotor (Beckman Instruments, Palo Alto, CA, USA). The fraction collected at the middle of the gradient was further purified by centrifugation at 67 000 ***g*** for 20 min (Beckman Optima TLX Ultracentrifuge, fixed Beckman TLA 100·4 rotor). Purified BMs were aliquoted, frozen in liquid N_2 and stored at − 80 °C.

Orientation assay

Membrane vesicle orientation was assessed using a previously published method (Illsley *et al.* 1990) and involved measuring the binding of concanavalin A–fluorescein isothiocynate (Con A–FITC) to the membrane vesicle fractions. To determine specific membrane binding, 0·5 mol/l methyl-α-D-mannopyranoside (which specifically displaces bound concanavalin A to α-mannosyl and α-glucosyl residues on the external membrane surfaces) was added. Vesicle lysis was carried out by three freeze (liquid N_2) and thaw (at 37 °C) cycles. The fraction of right-side-out vesicles was taken as the ratio of Con A–FITC binding in the presence and absence of methyl-α-D-mannopyranoside, measured before and after lysis. Con A–FITC was measured in a Perkin Elmer LS 50B Luminescence Spectrometer using a 515 nm emmission cut-on filter (slit width 20) and 490 nm excitation cut-on filter (slit width 15).

Alkaline phosphatase assay

The MVM marker, alkaline phosphatase, was assayed using a previously published method (Kelly & Hamilton 1970, Lafond *et al.* 1988, Illsley *et al.* 1990). In brief, a 50 µl sample (diluted in 0·02 mol/l NaOH) was incubated with 200 µl assay mixture (2 mg/ml *p*-nitrophenyl phosphate, 25 mmol/l sodium borate (pH 9·8), 2 mmol/l $MgCl_2$) for 30 min at 37 °C and the reaction stopped with 600 µl 0·25 mol/l NaOH. The absorbance was measured at 405 nm. Standards ranged from 0·25 to 0·004 µmol/ml.

Dihydroalprenolol binding assay

BM purity was determined using a modified dihydroalprenolol binding method (Williams *et al.* 1976). Placental homogenate or enriched vesicles (10–50 µg protein) were incubated in the presence of 10 nmol/l [^{3}H]dihydroalprenolol ([H^3]DHAP, Amersham, Amersham, Bucks, UK) in a total volume of 100 µl incubation buffer (50 mmol/l Tris–HCl, 10 mmol/l $MgCl_2$, pH 7·4) for 15 min at 37 °C. DL-Propranolol (10 µmol/l) was used to determined non-specific binding. After incubation, preparations were vacuum filtered on 0·45-µm filters through a Millipore 96-well Multiscreen plate (Bedford, MA, USA), which separated free radioactivity from bound. Filters retaining bound radiation were punched out, dried, placed in Optiphase 'HiSafe' 3 scintillant (Wallac Scintillation Products, Turku, Finland) and counted on a Liquid Scintillation Spectrometer (Model 1409, Wallac). Specific binding was determined by subtracting the non-specific binding from the total counts bound. [^{3}H]DHAP-specific binding to each of the fractions was represented as mmol [^{3}H]DHAP per µg membrane protein.

Effect of PTH(1–34) and PTHrP peptides on calcium efflux

The uptake of calcium by placental vesicles was measured by modification to a standard filtration method (Donowitz *et al.* 1987). Membrane vesicles (50–100 µg protein for BM and 15–20 µg protein for MVM vesicles) were preloaded with incubation buffer (250 mmol/l sucrose, 30 mmol/l Tris, 10 mmol/l Hepes, 5 mmol/l $MgCl_2$, pH 7·4), $CaCl_2$ (as required), and 5 µCi calcium-45 (DuPont NEN, Boston, MA, USA) in a total volume of 100 µl. The preload was carried out by freezing the above mixture and vesicles in liquid N_2 and thawing in an ice bath. Preloaded vesicles were filtered under vacuum on 0·45 µm Duropore membrane filters (Millipore Corporation) and washed three times with filtration buffer (250 mmol/l sucrose, 50 mmol/l Tris, pH 7·4). Incubation buffer containing peptide was then added to BM and MVM vesicles (with or without PTH(1–34), PTHrP(1–34) and PTHrP(67–94) at concentrations up to 50 nmol/l) and shaken continually at 37 °C for 30 min. In separate experiments in BM vesicles only, PTH(1–34), PTHrP(1–34) and PTHrP(67–94) (50 nmol/l) were incubated (at 37 °C for 30 min) in the presence and in the absence of the PTH/PTHrP receptor antagonist, PTHrP(7–34) (50 nmol/l). The plate was again filtered under suction and rinsed three times. The plate was dried in a 37 °C incubator cabinet before filters were punched out into scintillation vials containing Optiphase 'HiSafe' 3 scintillant using a Multiscreen Punch Assembly (Millipore Corporation) and counted on a Liquid Scintillation Spectrometer (Model 1409, Wallac).

Protein assay

The protein content of each of the membranes was determined by the protein dye-binding method described by Bradford (1976) using bovine serum albumin as a reference standard.

Statistical analyses

Statistical computations were performed using a commercially available statistical analysis package (Statgraphics, STSC, Rockville, MD, USA). The homogeneity of data was tested using Bartlett's test ($P<0·05$). Subsequently, two sample comparisons were analysed using Student's

Table 1 Representation of various membrane vesicle parameters: orientation, alkaline phosphatase activity (MVM marker) and dihydroalprenolol binding (BM marker). Orientation was calculated as % right side out (percent correct orientation). Alkaline phosphatase and dihydroalprenolol were determined as fold enrichment with respect to the initial homogenate. All data are expressed as means ± S.E.M. ($n=8$)

	Membrane vesicles	
	Microvillous	Basal
Assay		
Orientation (% right side out)	99·0 ± 0·3	102·4 ± 1·7
Alkaline phosphatase (fold enrichment)	12·2 ± 2·8	4·3 ± 0·9
Dihydroalprenolol binding (fold enrichment)	1·7 ± 0·7	12·9 ± 6·0

t-test, and three or more data sets were analysed using analysis of variance (ANOVA) with *post-hoc* analysis using a Newman–Keuls test. Statistical significance was indicated by $P<0·05$. Data are expressed as means ± standard error of the mean (S.E.M.). Because of the variable responses in calcium efflux between placentae, the calcium data were calculated as 'percent of control'.

Results

Orientation and membrane markers

Orientation and membrane markers were used to assess the configuration and fractionation of the respective membrane preparations. Both MVM (99·0 ± 0·3%) and BM (102·4 ± 1·7%) were approximately 100% in the correct or right-side-out orientation (Table 1). Alkaline phosphatase activity (an MVM marker) showed a three-fold increase in MVM relative to BM (Table 1). Table 1 also shows that dihydroalprenolol (a BM marker) binding was six-fold greater in the BM preparations with respect to that seen in the MVM fractions.

Effect of exogenous calcium and magnesium on the preload procedure

Dose–response curves were used to investigate whether calcium or magnesium had any effect on the uptake of calcium during the preload procedure. Calcium or magnesium were preloaded at various concentrations and incubated for 15 min at 37 °C. There was no significant difference between the two membrane vesicle preparations, with an approximate 1·5-fold increase in radioactive calcium uptake with either calcium or magnesium. On the basis of our data, 0·4 mmol/l calcium and 10 mmol/l magnesium were used in all subsequent experiments.

Effect of PTH(1–34) and PTHrP peptides on calcium efflux

PTH(1–34), PTHrP(1–34) and PTHrP(67–94) at various concentrations were added to the solution surrounding the preloaded vesicles and incubated for 15 min at 37 °C. None of the peptides examined had a significant effect on calcium efflux in the MVM fractions (Fig. 1). PTHrP(67–94) showed no significant increase in calcium efflux in

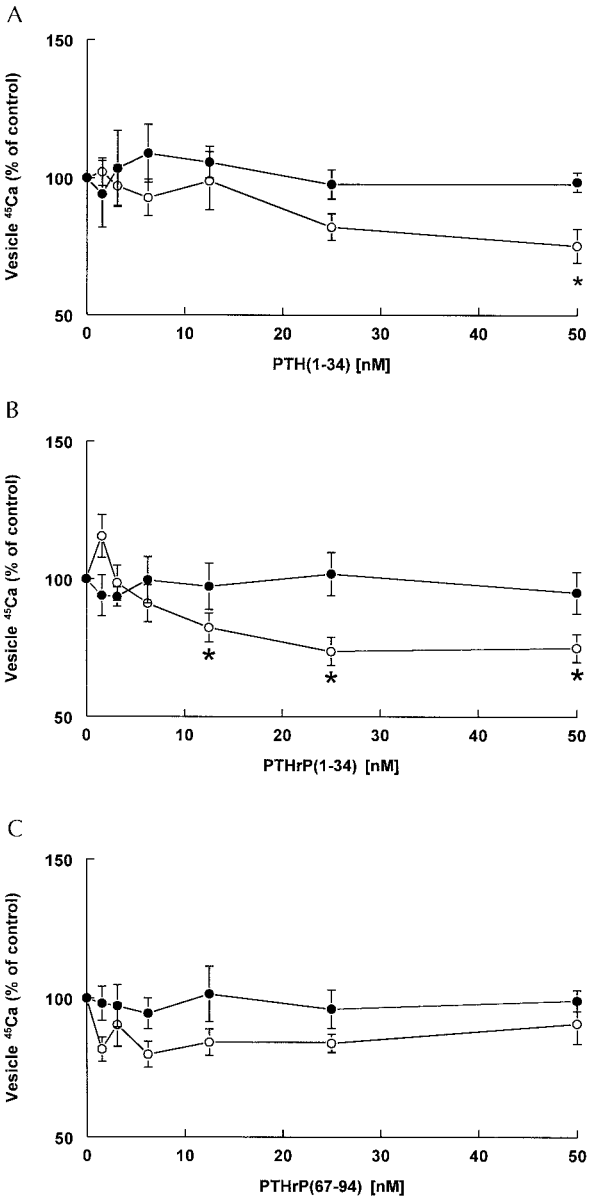

Figure 1 Effects of PTH(1–34) (A), PTHrP(1–34) (B) and PTHrP(67–94) (C) on vesicle calcium-45 (^{45}Ca) in BM (○) and in MVM (●) vesicles. Vesicle calcium-45 is calculated as % of time zero control. Membrane vesicles were preloaded (with 0·4 mmol/l Ca^{2+} and 10 mmol/l Mg^{2+}) and then incubated in the presence of varying concentrations of PTH(1–34) and PTHrP peptides for 15 min at 37 °C. At 50 nmol/l PTH(1–34) and from 12·5 nmol/l PTHrP(1–34) there was a significant efflux, as indicated by a decrease from basal in vesicle calcium-45 in BM (*$P<0·05$ from zero dose, ANOVA). Data are expressed as means ± S.E.M. ($n=8$).

Table 2 Effect of PTHrP/PTH receptor antagonist PTHrP(7–34) on vesicle calcium-45 (^{45}Ca) (pmol/h per mg protein) in BM vesicles in the presence of 50 nmol/l PTH(1–34), PTHrP(1–34) and midmolecule PTHrP(67–94). BM vesicles were preloaded (with 0·4 mmol/l Ca^{2+} and 10 mmol/l Mg^{2+}) and then incubated in the presence of PTH(1–34) or the PTHrP peptides, with and without PTHrP(7–34) (50 nmol/l), for 15 min at 37°C. Student's paired *t*-tests were performed between control and presence of PTHrP(7–34) for the various peptide combinations. In the presence of the PTHrP/PTH receptor antagonist PTHrP(7–34), there was a reduction in efflux (indicated by a decrease in vesicle calcium-45) in response to PTH(1–34) and PTHrP(1–34). Data are expressed as means ± S.E.M. (n=8)

	Vesicle ^{45}Ca (pmol/h per mg protein)	
	Control	PTHrP(7–34)
Peptide		
No peptide	225 ± 25	274 ± 45
PTH(1–34)	199 ± 23	242 ± 29*
PTHrP(1–34)	169 ± 14	230 ± 15**
PTHrP(67–94)	237 ± 22	275 ± 36

Significant reductions in efflux compared with control: *P 0·01, **P 0·006.

BM. At 50 nmol/l PTH(1–34) and from 12·5 nmol/l PTHrP(1–34) there was a significant efflux of calcium from BM compared with basal efflux without peptide (P<0·05, n=8, ANOVA; Fig. 1).

Effect of PTH/PTHrP receptor antagonist on calcium efflux

The common PTH/PTHrP receptor antagonist, PTHrP(7–34), was also added to the solution surrounding the preloaded vesicles, to investigate its effects on efflux of calcium-45 (pmol/h per mg protein) from BMs in the presence of PTH(1–34), PTHrP(1–34) and PTHrP(67–94) (Table 2). At 50 nmol/l, PTH(1–34) and PTHrP(1–34) caused significant efflux of calcium from the BM (P<0·01 and P<0·006 respectively, n=8, Student's *t*-test; Table 2). Addition of the PTH/PTHrP receptor antagonist, PTHrP(7–34), significantly reduced the calcium efflux observed in the BM vesicles in the presence of PTH(1–34) (P<0·01) and PTHrP(1–34) (P<0·006). The midmolecule, PTHrP(67–94), in the absence or presence of the receptor antagonist had no effect on calcium efflux from BMs.

Discussion

The syncytiotrophoblast represents the primary interface between the maternal and fetal circulations in the placenta. The use of simultaneous preparations of MVMs (maternal facing) and BMs (fetal facing) from term human placental syncytiotrophoblast provides a useful tool with which to explore the regulation of calcium efflux across the placenta (Smith *et al.* 1974). Before the recognition of midmolecule PTHrP peptides as regulators of placental calcium transport, it was reported that PTH influenced phosphate transport across purified placental MVMs and increased cAMP accumulation (Brunette *et al.* 1989). Receptors for PTH were implicated in both MVM and BM, but adenylate cyclase activity was found exclusively in the placental BMs (Smith *et al.* 1974, Lafond *et al.* 1988). More recent investigation of G-proteins in these membrane vesicles indicates that the syncytiotrophoblast BM vesicle preparation contains all the elements necessary to allow activation of the PTH/PTHrP receptor (el Mabrouk *et al.* 1996). Calcium transport across the MVM of the human placental syncytiotrophoblast is assisted by a specific membrane carrier and the movement of calcium through the syncytiotrophoblast is aided by calcium-binding proteins. In the MVM, calcium uptake is reported to be time dependent and it was found that calcium entered the cell via two distinct transporters and was bound to internal sites (Kamath *et al.* 1992). High-affinity calcium pumps in human and rat placentae were found to reside in the BM portion of the syncytiotrophoblast and it was concluded that these pumps played a part in the transport of calcium from the maternal to the fetal circulation (Borke *et al.* 1989). Although little information is available on the efflux of calcium from the syncytiotrophoblast, the presence of a pump with characteristics similar to those of a Ca^{2+}–Mg^{2+}-ATPase pump has been suggested to be present, with greater activity in the BM compared with the MVM, suggesting the possibility of a role in calcium transport (Fisher *et al.* 1987, Borke *et al.* 1989, Lafond *et al.* 1991). Thus the simultaneous membrane vesicle preparation is a useful model system for the study of the role of PTHrPs in the movement of calcium across the placental syncytiotrophoblast.

In this study, the vesicles produced were in the correct or right-side-out orientation and the specific membrane markers demonstrated that MVMs were successfully isolated from BMs, indicating that this system was optimized to investigate calcium transport across the respective syncytiotrophoblast membranes. With these simultaneously prepared MVMs and BMs, we have unequivocally demonstrated that both PTH(1–34) and PTHrP(1–34) regulate calcium transport across the BM, but not the MVM, of the syncytiotrophoblast. Although the relative potencies remain to be established, PTHrP(1–34) in BM vesicles was found to stimulate significant calcium efflux at a dose of 12·5 nmol/l, whereas PTH(1–34)-stimulated efflux was significant at 50 nmol/l. The PTHrP midmolecule had no significant effect on calcium efflux at either membrane. Furthermore, we were able to demonstrate that the PTH/PTHrP receptor antagonist, PTHrP(7–34), antagonized the calcium efflux from BMs in response to both PTH(1–34) and PTHrP(1–34), indicating a receptor-mediated event. The data presented in this paper indicate that PTH(1–34) and PTHrP(1–34), acting through the PTH/PTHrP receptor, can stimulate

calcium transport across the BM, but not the MVM, of the human syncytiotrophoblast. In this way, PTH(1–34) and PTHrP(1–34) may contribute to the overall maintenance of calcium transfer across the placenta, which has previously been reported in the literature to be controlled, in the main, by midmolecule PTHrP peptides (Rodda *et al.* 1988, Care *et al.* 1990).

In contrast to the sheep studies carried out earlier in gestation (Rodda *et al.* 1988), the midmolecule, PTHrP(67–94), had no effect on calcium transport across human MVMs and BMs. Previous studies of the effects of PTH or N-terminal PTHrP on placental calcium transport have been controversial, and confounded by the use of inappropriate models. For example, the presence of intact fetal parathyroids, kidneys and skeleton, in addition to the use of a rat decapitation model, complicate the interpretation of the data (Robinson *et al.* 1989, Barlet *et al.* 1990, Shaw *et al.* 1991). The presence of responsive PTH/PTHrP receptors in the human placenta, and specifically on the BMs of the syncytiotrophoblast (Smith *et al.* 1974, Lafond *et al.* 1988, el Mabrouk *et al.* 1996), together with the present observations, are indicative of a potential role of N-terminal PTH and PTHrP in stimulating human placental calcium transport. However, the effects observed in this *in vitro* human model were modest, and may represent a modulating effect either of local PTHrP or of circulating PTH. Although knockout studies indicated that the PTH/PTHrP receptor was not necessary for placental calcium transport (Kovacs *et al.* 1996), N-terminal PTH or PTHrP activity may contribute to alternate calcium transport mechanisms apparent in the animals null for PTHrP or calcium sensor receptor in which placental calcium transport is not completely ablated (Kovacs *et al.* 1996, 1998).

The physiological contribution of the effect of N-terminal PTH or PTHrP on human placental membrane vesicles remains to be fully elucidated and possible differences in the control of placental calcium transport arising from gestational age and species differences cannot be disregarded. Further functional studies in animals null for PTHrP, PTH and PTH/PTHrP and calcium sensor receptors should contribute significantly towards understanding the relative contributions of all these mechanisms to placental calcium transport during fetal development. These approaches would be complemented by studies characterizing the second-messenger pathways involved and the development of an *in vitro* placental tissue explant system in which the specific mechanisms of PTHrP action in the placenta could be explored.

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