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Effect of Parathyroid Hormone on PO_4 Transport through the Human Placenta Microvilli

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ABSTRACT. The transport of phosphate (PO_4) through the placenta is a secondary active phenomenon whose control mechanisms are unknown. In this study, we investigated whether PTH, the main hormone regulating PO_4 transport in the kidney and gut, has a similar role in the placenta. Incubation of normal term human placenta fragments for 1 min with PTH increased the cAMP content of the tissue by 285%. A dose-response curve of the effect of the hormone showed that the cAMP accumulation reached a maximal level with 3.5×10^{-8} M PTH. Incubation of the placenta fragments with 10^{-4} M di-butyryl cAMP resulted in a significant decrease in the PO_4 uptake by the brush border membranes prepared from these fragments. Increasing concentrations of di-butyryl cAMP from 0 to 10^{-3} M significantly decreased the PO_4 uptake from 0.29 ± 0.02 to 0.22 ± 0.01 pmol/μg/20 s. Similarly, incubation of the placental tissue with PTH resulted in a comparable decrease in the PO_4 uptake by the corresponding brush border membrane vesicles. In contrast, direct incubation of brush border membranes with the hormone did not influence PO_4 uptake. It is concluded that PTH probably regulates the PO_4 transport through the placenta syncytiotrophoblast cell through cAMP mediation. Because adenylate cyclase is located in the basal plasma membrane, it is likely that only the fetal hormone is implicated in this process. (*Pediatr Res* 25:15–18, 1989)

Abbreviations

Db, di-butyryl
BBM, brush border membranes
BTM, 1-bromotetramisol
PO_4, phosphate

In mammalian species, the concentration of PO_4 in fetal plasma is higher than that in maternal plasma. As transplacental potential difference is close to 0 (1) or negative (2, 3), it is accepted that PO_4 transport through the placenta is an active phenomenon.

We recently described inorganic PO_4 uptake by placental BBM vesicles, using the rapid filtration technique (4). The results indicated that PO_4 was transported through this membrane according to a secondary active mechanism, using Na^+ gradient as a source of energy. However, the regulation of PO_4 delivery to the fetal circulation is poorly understood.

In the present study, we examined whether PTH which, in the kidney and gut, represents the main PO_4 transport regulating hormone, plays a similar role in human placenta. We incubated placenta fragments with PTH, and studied the effect of the hormone on PO_4 transport through the BBM. Because cAMP is one of the putative cellular mediators of PTH, we determined whether incubation of placental tissue with PTH results in a cAMP accumulation and whether incubation with cAMP can reproduce the effects of PTH on PO_4 transport.

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MATERIALS AND METHODS

Incubation of placenta tissue with PTH for cAMP production. The experiments were performed with normal term human placentas. Immediately after delivery, the placentas were perfused with saline solution containing NaCl 150 mM and KCl 5 mM. Tissue fragments were taken from the central part of the placenta, after 1-cm thick slices below the amniotic and the chorionic surface were eliminated. These fragments were suspended in Krebs-Henseleit solution and oxygenated 30 min at 37°C to deplete the tissue of native cAMP. Methyl-3-isobutyl-xanthine 1 mM (final concentration) was used with or without PTH. After a 3-min incubation (unless otherwise specified), the incubation was stopped by diluting the mixture with perchloric acid 1 M, final concentration. The suspension was sonicated and frozen at −70°C until cAMP measurement.

cAMP measurement. cAMP was measured in the supernatant of the sonicated fragments of tissue after a 15-min centrifugation at 5000 rpm. Proteins were measured in the pellet. All the cAMP assays were performed in duplicate, simultaneously in tissues incubated with and without PTH, using the Immuno Nuclear RIA test (ICN Biochemicals Inc., Costa Mesa, CA).

Incubation of placenta tissue with PTH for PO_4 uptake experiments. These incubations were similar to those performed in cAMP measurement experiments. However, because of the more complex cascade of events between the eventual binding of the hormone and the modification of PO_4 uptake by the membranes, some additional precautions were taken. In particular, incubation with PTH lasted 45 min at 20°C instead of 3 min at 37°C, the hormone was protected by the addition of 1% BSA; 1 mM ATP and a regenerating system of ATP were included in the medium (phosphocreatine 20 mM, creatine kinase 100 U/ml), and finally protein phosphorylation was protected by phosphatase inhibitors, *i.e.* KF 10 mM and BTM 1 mM. Incubation was performed at room temperature to minimize PTH deterioration and stopped by rapid filtration of the suspension through gauze in an ice-cooled beaker. The filtrate was then centrifuged at 100,000 × g as described below for BBM preparation.

Incubation of brush border membranes with PTH. BBM suspended in Tris-HEPES 10 mM pH 7.0 and Mannitol 300 mM were incubated 5 min at 35°C in a hypotonic solution containing Tris-HEPES 5 mM pH 7.0, $MgCl_2$ 10 mM, KF 10 mM, phosphocreatine 20 mM, creatine kinase 100 U/ml, Mg ATP 10 μM, to which PTH 0.35 μM was or was not added. The suspension was then re-suspended in 10 volumes of Tris-HEPES 10 mM, pH 7.0, Mannitol 300 mM, KF 10 mM, and centrifuged at 27,000 × g for 15 min at 4°C. The pellet was collected, suspended

in Tris-HEPES 10 mM, Mannitol 300 mM, and kept for 1 h at room temperature to permit vesiculization before the PO_4 uptake experiments.

Incubation of placenta tissue with Db cAMP. Tissue fragments were prepared as for incubation with PTH. After the preincubation in Krebs-Henseleit solution 30 min at 30°C for depletion of cAMP, Db cAMP was added to the suspension at 10^{-4} M concentration unless otherwise specified. Also added to the incubation medium were: BSA 0.5%, theophylline 1 mM, phosphatase inhibitors (BTM 1 mM and KF 10 mM), ATP 1 mM and a regenerating system, *i.e.* phosphocreatine 20 mM, creatine kinase 100 U/ml. Incubation was performed at room temperature for 45 min with a magnetic stirrer. The suspension was then filtered through gauze, ready for membrane preparation.

Preparation of BBM. Placental BBM were isolated by a previously described procedure (4) which is a modified version of the method described by Smith *et al.* (5). The main steps are as follows: after the fragment suspension was filtered with six layers of gauze cloth, the filtrate was centrifuged at 100,000 × g for 60 min at 4°C. The pellet was homogenized with a Teflon Potter homogenizer (Elvejhem) at 2,000 rpm, and resuspended in 15 ml Tris-HEPES 20 mM, pH 7, Mannitol 300 mM, added with $MgCl_2$ 10 mM. The mixture was stirred with a magnetic bar, 20 min at 4°C and centrifuged at 3,000 × g for 10 min. The supernatant containing the BBM was decanted and centrifuged at 27,000 × g 10 min at 4°C. The membrane pellets were suspended in Tris-HEPES 10 mM, pH 7.0, Mannitol 300 mM, washed once, and resuspended in the same medium with a final protein concentration of approximately 20 mg/ml. Uptake experiments were started 1 h after preincubation in order to permit vesiculization. Whole placenta homogenate was prepared directly from fragments of the central part of the perfused placenta. The purity of the BBM was monitored by assaying alkaline phosphatase, the marker enzyme for BBM, and the Na^+, K^+ ATPase, the marker enzyme for basal cytosol membrane and possibly capillary membranes. Alkaline phosphatase enrichment was 30.8 ± 4.6 and Na^+, K^+ ATPase was 6.4 ± 1.8.

PO_4 uptake. PO_4 uptake measurements were made by the rapid filtration technique using Millipore filters (0.45 μm). (Millipore Continental Water Systems, Schenectady, NY). Uptake was initiated by mixing 5 μl of the membrane suspension (100 μg protein) with 25 μl of incubation medium containing labeled ^{32}P. The composition of this medium was (final concentration) Mannitol 100 mM, NaSCN (or KSCN) 100 mM, Tris-HEPES 10 mM at pH 7.0, and ^{32}P Na^2 HPO_4 0.04 mM, specific activity 1.8 μCi/nmol. Incubation was performed at 35°C.

Na uptake. was measured in a similar fashion, except that $^{22}NaSCN$ (0.3 ml/mmol) replaced NaSCN and Na^2PO_4 replaced $^{32}PNa^2HPO_4$.

Chemical measurement. The protein content of the total tissue or membrane suspensions was determined by the method of Lowry *et al.* (6) using BSA as standards. Alkaline phosphatase activity was measured according to the method of Kelly and Hamilton (7). Na^+, K^+ ATPase activity was estimated using the technique of Post and Sen (8) in which the liberated phosphate was measured with the method described by Atkinson *et al.* (9).

Material. ^{32}P-labeled phosphoric acid was purchased from New England Nuclear (Boston, MA), and bovine PTH (1–34) synthetic from Peninsula Laboratories, Inc. (Belmont, CA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Statistical analysis. All values are expressed as mean ± SEM. Results are evaluated by the unpaired Student's *t* test.

RESULTS

Effect of PTH on cAMP content of the tissue. Figure 1 depicts the influence of incubation time on the cAMP content of the placental tissue when the fragments are incubated in the presence and the absence of 0.35 μM PTH. The baseline value of cAMP content in crude tissue was 20.5 ± 3.2 pmol/mg of protein. After

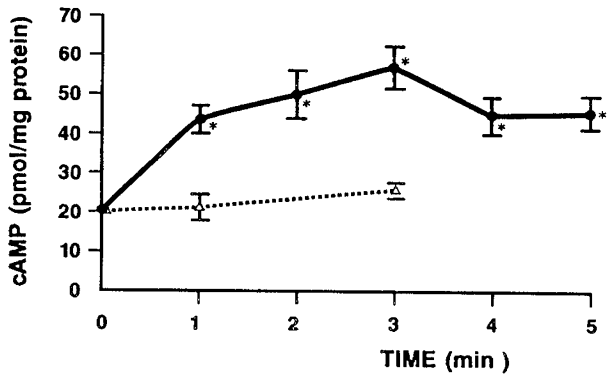

Fig. 1. Effect of time on incubation of placental fragments with PTH 0.35 μM on cAMP accumulation in the tissue. The fragments were incubated in Krebs-Henseleit solution containing methyl-3-isobutyl-xanthine 1 mM, with (●) or without (△) 0.35 μM PTH, before cAMP measurement. Mean ± SE, $n = 5$. *, $p < 0.05$.

1 min of incubation, stimulation was already present, resulting in an increase in the concentration of cAMP to 57.1 ± 5.2 pmol/mg, which was significantly different from both the initial level and the value measured in the control tissue incubated without PTH ($p < 0.01$).

Figure 2 shows the dose-response curve of the effect of PTH on the formation of cAMP. The cAMP intracellular accumulation in the placental fragments increased with the concentration of PTH. This increase in cAMP was already significant with PTH 3.5×10^{-9} M ($p < 0.01$). Maximal stimulation was obtained with PTH 35×10^{-9} M.

Effect of incubation of placental tissue with Db cAMP on PO_4 uptake by BBM vesicles. Figure 3 represents the time-course of PO_4 uptake by the vesicles when the original placental tissue was preincubated with and without 10^{-4} M Db cAMP. After 10 and 20 s of incubation, the mean initial uptake tended to be lower in vesicles pretreated with cAMP, the difference between the two kinetics becoming significant at 1 min ($p < 0.05$) and 2 min ($p < 0.01$, $n = 3$). After 10 min of incubation, the intravesicular PO_4 accumulation returned to the same values in the two series of preparations.

A dose-response curve is presented in Figure 4. Increasing concentration of Db cAMP from 10^{-9} to 10^{-3} M in the preincubation medium of the placental tissue progressively decreased the mean value of the initial PO_4 uptake from 0.29 ± 0.02 to 0.22 ± 0.01 pmol/μg/20 s by the corresponding BBM vesicles. This fall of PO_4 uptake was significant at any of the concentrations of Db cAMP used.

Effect of incubation of placental tissue with PTH on PO_4 uptake by BBM vesicles. Preincubation of placental tissue with 3.5×10^{-1} μM PTH resulted in a decrease in PO_4 uptake by the subsequently isolated BBM. Figure 5 represents a time-course of PO_4 uptake by membranes from fragments of placenta incubated with and without PTH. In each of the three experiments whose mean values are shown in this Figure 5, incubation with PTH resulted in a significant decrease in PO_4 uptake by the membranes at any time of incubation between 10 and 120 s. In contrast (not shown in Fig. 5), incubation of placental tissue with PTH did not influence Na^+ uptake by the vesicles.

Effect of incubation of BBM with PTH. Incubation of the purified membranes directly with PTH did not influence the PO_4 uptake by the vesicles.

DISCUSSION

During gestation, in any species studied, *i.e.* rat (10, 11), calf and sheep (12–14), guinea pig (15, 16), and human (17), phosphatemia is higher in the fetus than in the mother, particularly at the end of gestation. Despite this concentration difference

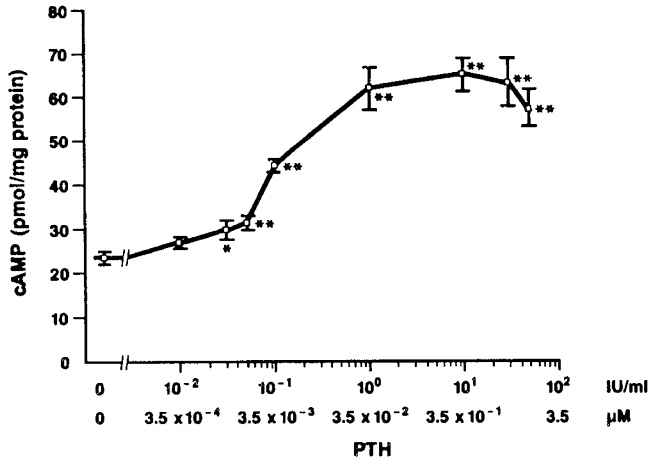

Fig. 2. Dose-response curve of the effect of 3-min incubation of placental fragments with PTH on cAMP accumulation in the tissue. Mean ± SE, n = 3–10, depending on the PTH concentrations. * $p < 0.05$; ** $p < 0.01$.

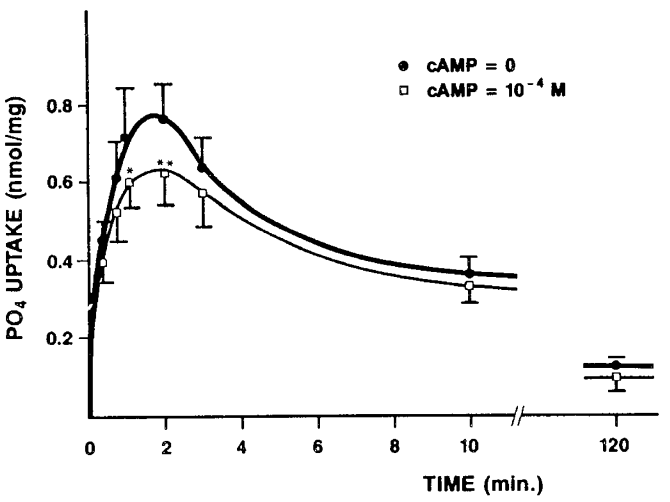

Fig. 3. Effect of incubation of placental tissue with cAMP 10^{-4} on the time course of PO_4 uptake by the BBM subsequently isolated from this tissue. Tissue fragments were incubated 45 min at 20°C in Krebs-Henseleit solution added with BSA 0.5%, theophylline 1 mM, bromotetramisole 1 mM, KF 10 mM, ATP 1 mM, phosphocreatine 20 mM and creatine kinase 100 U/ml, in the presence (□) or absence (●) of cAMP 10^{-4} M. Mean ± SE, n = 3. * $p < 0.05$; ** $p < 0.01$.

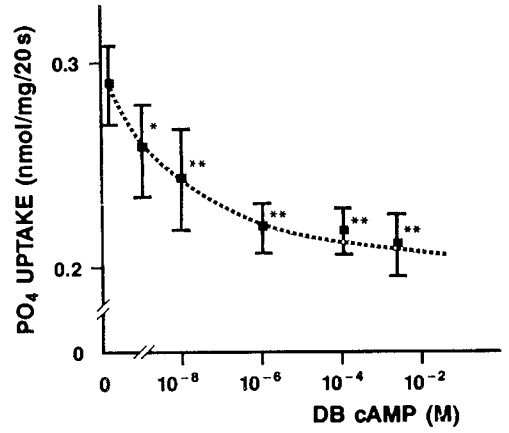

Fig. 4. Dose-response curve of the effect of incubation of placental tissue with cAMP on the 10 s PO_4 uptake by the BBM subsequently isolated from the tissue. Mean ± SE, n = 7. * $p < 0.05$; ** $p < 0.01$.

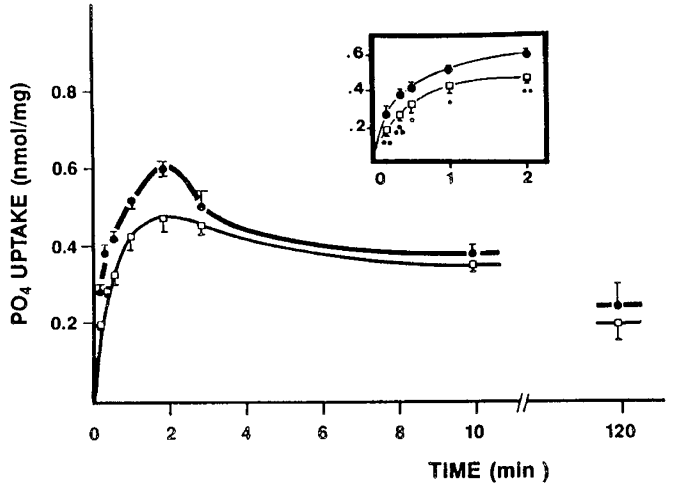

Fig. 5. Effect of incubation of placental tissue with PTH 0.35 µM on the time-course of PO_4 uptake by the BBM subsequently isolated from this tissue. Tissue fragments were incubated 45 min at room temperature in Krebs-Henseleit solution added with 1% BSA, ATP 1 mM phosphocreatine 20 mM, creatine kinase 100 U/ml, KF 10 mM, BTM 1 mM and with (□) or without (●) PTH. After the incubation, the tissue was filtrated, centrifuged, and membranes were prepared from the sediment as described in "Materials and Methods." Mean ± SE, n = 4. *Inset*: the detail of the initial uptake of PO_4. * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$.

across the placenta, and the absence of favorable potential difference (1–3) ^{32}P transfer increases until late in gestation (10, 15, 18). It is therefore believed that PO_4 transport through the placenta is an active phenomenon. Although this transport is modulated by factors such as PO_4 and Ca^{2+} concentrations on the fetal side (19) or Na^+ concentrations, pH, and amino acids on the maternal side (4, 20), it is probable that some hormonal control is implicated in its regulation. In the human, immunoreactive PTH has been detected in the fetal parathyroid gland at 10 wk of gestation (21). Using a sensitive cytochemical bioassay, Allgrove *et al.* (22) measured and compared the immunoreactive *versus* the bioactive form of the hormone in the fetal blood. These authors reported that, whereas the immunoreactive hormone was relatively low, the bioactive form was 5-fold higher than that in the mother's blood.

The effect of PTH injected in sheep fetus was investigated by Barlet *et al.* (23), who reported a dramatic decrease in fetal plasma PO_4 concentrations with a peak of action at 180 min after the injection. This drop may be due, as in the adult, to a shift of PO_4 to the intracellular compartment, or to a renal clearance. Whether the placenta contributes to this hypophosphatemia is unknown.

Placental tissue has receptors for PTH (24), and it was therefore of interest to investigate whether this hormone influences PO_4 transport in the placenta as it does in kidney and gut.

Our present results demonstrate that PTH enhances cAMP formation and that both the hormone and its messenger, when incubated with the placental tissue, depress PO_4 uptake by the isolated BBM. It is therefore probable that PTH plays a regulating role in PO_4 transport from the mother to the fetus independent of Na^+ transport. In contrast, PTH directly added to the BBM does not have any influence on PO_4 uptake. These observations support the hypothesis that PTH needs the mediation of intracellular messengers to be efficient.

It is not yet established whether it is PTH from the fetal, maternal, or both compartments which influences PO_4 transport. Indeed, Whitsett (25) reported that adenylate cyclase was exclusively located in the basal cytoplasmic membrane of the syncy-

tiotrophoblast cell and therefore on the fetal side of the cell. This finding suggests that only fetal PTH directly regulates the PO_4 transport. In this case, the maternal hormone could have influence only through the synthesis of $1,25(OH)_2D_3$, which passes to a certain degree across the placental barrier (26, 27). More information on the exact sites of receptors is needed to understand the respective roles of fetal and maternal PTH in the regulation of PO_4 transport through the placenta.

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