Circulating levels of immunoreactive parathyroid hormone-related protein and intact parathyroid hormone in human fetuses and newborns

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Undetectable or extremely low levels of circulating immunoreactive parathyroid hormone (PTH) have been reported in human newborns while PTH bioactivity was high. This prompted the hypothesis that the fetal calcemic hormone might be PTH-related protein. The purpose of this study was to measure circulating immunoreactive PTH-related protein in human fetuses and newborns in order to investigate this hypothesis. Parathyroid hormone-related protein (PTHrP(1–86)) and intact PTH were measured using two-site immunoradiometric assays in plasma obtained by cordocentesis from 23 fetuses (19–33 weeks of gestation), from 17 newborns at term (38–41 weeks), from their mothers and from 22 normal women of reproductive age. Plasma PTHrP was detectable in all but one of the fetuses and newborns and in all the mothers and the controls. The mean level was similar among fetuses (19–33 weeks) (0.43 ± 0.18 pmol/l), newborns (0.48 ± 0.12), mothers (0.48 ± 0.14) and normal controls (0.46 ± 0.09). Plasma PTH was found to be significantly higher in fetuses at mid-gestation (1.0 ± 0.99 pmol/l) than in the newborns (0.22 ± 0.21) (p < 0.0025); maternal PTH was significantly higher compared to fetal level at mid-gestation (2.1 ± 1.0, p < 0.01) as well as at term (2.69 ± 1.40, p < 0.001). In the control women PTH was 3.07 ± 1.25 pmol/l. These results showed that plasma amino-terminal PTHrP-(1–86)) is detectable during the second half of human fetal life and its level remains unchanged during this period of time, in contrast to changing levels of fetal plasma PTH. The relatively low PTHrP-(1–86) level that we found in the newborns is not responsible for the high PTH-like bioactivity found by some investigators in cord blood at term.

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Parathyroid hormone-related protein (PTHrP) is produced by many malignant tumors, causing the syndrome of humoral hypercalcemia of malignancy. This syndrome is the result of a parathyroid hormone (PTH)-like effect of PTHrP (1). Parathyroid hormone-related protein is also produced by a multitude of normal cells and many PTH-unlike effects of this protein have been elucidated recently (2, 3).

Parathyroid hormone-related protein is expressed temporarily in various fetal tissues during embryogenesis and is considered to play a role as a cellular growth and differentiation factor in fetal growth and development, acting in an autocrine or paracrine fashion (2, 3).

Fetal extracellular calcium concentrations higher than maternal have been documented in most mammals, including man (4, 5). This feto-maternal calcium gradient is maintained by a calcium-pump function of the placenta (6, 7). Experimental evidence suggests that fetal PTH is not the agent that controls the active placental calcium transport mechanism. In umbilical cord blood at term, immunoreactive PTH levels are extremely low or undetectable, while PTH-like bioactivity was found to be higher than in maternal blood (8, 9). This finding prompted the hypothesis that the humoral factor with PTH-like activity in the fetal circulation might be PTHrP. Mallette (10), reviewing the published pertinent experimental results, hypothesized that PTHrP is the fetal calcemic hormone responsible for the control of the placental calcium transport function. However, the actual source of the fetal PTHrP has not been established. Rodda et al. (11) have shown that in the fetal lamb, removal of the parathyroid glands abolished the feto-maternal calcium gradient. This finding indicates that, in this species, the main source of the fetal PTHrP is the fetal parathyroid gland; if this theory is correct, PTHrP should be detectable in fetal circulation. However, other evidence suggests that the placenta itself could be the source of fetal PTHrP and in this case the placental effect of PTHrP could be paracrine or autocrine (12). It would be
of interest, therefore, to know the actual levels of PTHrP in the fetal circulation. Measurements of PTHrP in plasma of human newborns reported recently gave conflicting results: Hillman et al. (13) found higher levels of hPTHrP(1–34) in cord blood of term and premature neonates compared to adults; Thiebaud et al. (14) found that PTHrP(1–86) levels in cord blood of human neonates were similar to the levels found in normal control non-pregnant women, but higher than in the corresponding pregnant mothers. The pregnant mothers, therefore, had lower PTHrP levels than non-pregnant women. In contrast, Khosla et al. (15) found low levels of PTHrP(1–34) in umbilical cord blood at term, comparable to normal adult levels. Finally, Seki et al. found higher neonatal cord than maternal serum levels of a carboxy-terminal immunoreactive molecular species of PTHrP (16).

In efforts to resolve these apparent inconsistencies regarding the circulating PTHrP in the human neonate, we measured immunoreactive PTHrP(1–86) in the cord blood of human neonates at birth and in fetal blood obtained by cordocentesis at mid-gestation. The results indicate that PTHrP is detectable in fetal blood during the second half of the human fetal existence, at concentrations similar to those of pregnant and normal non-pregnant women. The level of PTHrP in the fetal blood remains unchanged during the second half of the intrauterine life.

Material and methods

Subjects

We studied 22 normal young normocalcemic women (aged 20–42 years) not taking any medication and forty pairs of pregnant women and their fetuses or neonates. Fetal blood was obtained by cordocentesis from 23 fetuses between 19 and 33 weeks of gestation. In all cases fetal blood sampling was indicated for prenatal diagnosis of thalassemia. Only normal or heterozygous fetuses for β-thalassemia are included in this study. Cord blood was collected from the umbilical vein of 17 neonates at the time of delivery (38–41 gestational weeks). Eight of these neonates were born by caesarean section and nine after an uneventful labor. Venous blood was withdrawn from the mothers within a few minutes from the fetal sampling. The excess fetal blood obtained by cordocentesis and the neonatal and maternal blood obtained at the time of delivery was transferred immediately into heparinized tubes that contained a mixture of protease inhibitors and were kept in ice (tubes supplied by Nichols Institute); they were centrifuged within a few minutes and the separated plasma was stored at −80°C until it was assayed within a few days. Informed consent was obtained from all the women who participated in the study. The study was approved by the Scientific Committee of the hospital. In the rest of the text the name "fetuses" will signify fetuses between 19 and 33 weeks of gestational age and the name "newborn or neonates" will mean newborns at term (38–41 weeks) unless stated otherwise. Their mothers will be named mothers of fetuses and mothers of newborns, respectively.

Methods

Parathyroid hormone-related peptide was measured in plasma using a two-site immunoradiometric assay (IRMA) (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay uses two different polyclonal antibodies raised against synthetic human PTHrP and purified by affinity chromatography. The capture antibody binds only amino acid sequence 60–72 and is coupled to biotin. The signal (125I-labeled) antibody recognizes the N-terminal 1–40 region of the PTHrP molecule. Synthetic human PTHrP(1–86) was used as a standard. All standards were measured in triplicate and plasma samples in duplicate.

The detection limit of the assay was 0.1 pmol/l, defined as the mean ± 2sd of the zero standard (counts per min). Based on results for 18 assays, the intra- and interassay cv's of three quality-control pools with mean values of 0.5, 2.4 and 10.8 pmol/l were 6.6%, 4.5% and 2.5% and 10.6%, 7.6% and 4.3%, respectively. This IRMA is specific for PTHrP: it does not detect human PTH(1–84) or its fragments 1–34, 53–84 or 44–68. The assay detects equally human PTHrP fragments 1–72 and 1–86, while its 1–34, 37–74 and C-terminal fragments have been shown not to cross-react in the assay. Recovery of synthetic PTHrP(1–86) added to normal, maternal and neonatal plasma (obtained from heparinized tubes with antiproteases) at concentrations of 5, 2.5, 1.25 and 0.6 pmol/l ranged from 93 to 115%.

Parathyroid hormone(1–84) was measured by an IRMA kit (Nichols Institute). The detection limit of the assay was 0.2 pmol/l. The intra- and interassay cv's of two quality-control pools with mean values of 3.5 and 29.4 pmol/l were 3.2 and 2.8% and 4.6 and 4%, respectively.

Differences between the mean values of groups were statistically evaluated by the t-test. One-way ANOVA was used to evaluate possible differences among fetal, maternal and control mean plasma levels of PTHrP.

Results

Parathyroid hormone-related protein was detectable in the plasma of all normal subjects and mothers and in 39 of the 40 fetuses and neonates. In the control group it was found to be 0.46 ± 0.09 pmol/l (mean ± sd). In the fetuses it was 0.43 ± 0.18, which is not different from that of the newborns (0.48 ± 0.12). Thus, fetuses and newborns were combined in one group and the mean PTHrP plasma level in fetal plasma during the second half of fetal existence (N = 40) (19–41 gestational
weeks) was found to be $0.45 \pm 0.15$. No significant difference was found between arterial and venous cord plasma levels of PTHrP(1–86) determined separately in six neonates. In the mothers of fetuses who underwent transabdominal cordocentesis, plasma PTHrP was $0.51 \pm 0.16$, which is not different from that found in the mothers of the newborns during labor at term ($0.45 \pm 0.12$). Thus, the mean PTHrP in maternal plasma during the second half of pregnancy ($N = 40$) was $0.48 \pm 0.14$. These data are shown in Fig. 1. One-way ANOVA among the means of all the fetal–neonatal, maternal and normal control values of

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**Fig. 1.** Individual values of plasma parathyroid hormone-related protein (PTHrP) concentration of fetuses and newborns (left panel) and their mothers (right panel). The horizontal broken line indicates the detection limit of the assay (0.1 pmol/l).

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**Fig. 2.** Individual values of intact parathyroid hormone (PTH) plasma concentration of fetuses and newborns (left panel) and their mothers (right panel). The horizontal broken line indicates the detection limit of the assay (0.2 pmol/l).
plasma PTHrP showed that they were not different (F = 0.57). Plasma PTH in normal controls was 3.07 ± 1.29 pmol/l. Plasma PTH was determined only in 27 fetuses and neonates in whom sufficient plasma was available. It was undetectable in three of 10 fetuses and in nine of 17 newborns. A value of 0.08 pmol/l (median between zero and the detection limit) was assigned to the undetectable levels for the purpose of statistical calculations. In 10 fetuses plasma PTH was 1.0 ± 0.99, which is significantly higher than in the newborns (0.22 ± 0.21, p < 0.0025). In the mothers of fetuses undergoing transabdominal cordocenteses, plasma PTH was 2.1 ± 1.0, which is slightly lower compared to the value of 2.69 ± 1.40 found in the mothers of newborns during labor at term. Maternal mean PTH level was significantly higher compared to the fetal level at mid-gestation (p < 0.01) as well as at term (p < 0.001). These values are shown in Fig. 2. No correlation was found between paired fetal–maternal PTHrP or PTH values.

Serum Ca was 2.33 ± 0.16 mmol/l in normal controls. 2.50 ± 0.24 mmol/l in the mothers and 2.79 ± 0.23 mmol/l in the newborns (N = 12), which are significantly higher values than in both of the previous groups (p < 0.001).

Discussion

Experimental evidence indicates that PTHrP plays a role in the growth and development of many embryonic tissues of birds and mammals (2, 3, 7). During embryogenesis, spatial and temporal changes in the expression of PTHrP gene have been observed in various tissues where PTHrP acts as a tissue-specific cellular growth and differentiation factor, very likely in a paracrine or autocrine fashion (2, 3).

Serum total and ionized calcium concentrations in the infant at birth and in the second-trimester cord blood obtained by cordocentesis exceeds that of the mother, suggesting that there is an active placental transport of calcium from mother to fetus (4–6). The transcellular movement of Ca²⁺ across the syncytiotrophoblast is probably controlled by mechanisms similar to the mechanisms controlling the reabsorption of calcium in the distal nephron and the intestinal absorption of calcium (17).

The regulation of the Ca²⁺ concentrations in the fetal extracellular fluid requires the existence of a Ca²⁺-sensing apparatus located somewhere within the fetoplacental unit and a feedback mechanism by which the placental calcium pump is modulated. In humans after birth the acute regulation of the extracellular Ca²⁺ concentration depends on the secretion of PTH. There is a steep inverse relationship between PTH secretion and the extracellular ionized calcium concentration (17). The extracellular Ca²⁺ sensors seem to be Ca²⁺ (polycation)-binding receptors on the surface of the PTH-secreting parathyroid gland cells (17).

Rodda et al. (7) have demonstrated that, in fetal sheep, the relatively high serum Ca level falls after parathyroidecctomy of the fetus performed in utero. Administration of PTH(1–84), PTH(1–34) or PTHrP(1–34) to their in vivo sheep placental perfusion model was unable to stimulate placental calcium transport. In contrast, recombinant PTHrP, in addition to fetal lamb parathyroid extracts, was able to stimulate placental calcium transport in this model. These investigators concluded that PTH plays no role in the fetal plasma regulation of calcium. They suggested that the putative regulator of extracellular Ca²⁺ in the fetal sheep is probably PTHrP secreted by the fetal parathyroid glands (7). The lack of role of PTH in the fetus is indicated by the extremely low or undetectable levels of plasma immunoreactive PTH found in human newborns (8, 9). We confirmed here that immunoreactive PTH in cord blood at term is usually undetectable. However, we found that at mid-gestation fetal plasma PTH was measurable and significantly higher than in cord blood at term, although lower than maternal or normal adult levels.

According to the above-mentioned theory of Rodda, Cape and Martin (7), PTHrP should circulate in human fetal blood. We found that plasma PTHrP(1–86) is detectable at low concentration in fetuses and newborns between 19 and 41 weeks of fetal life. The mean fetal–neonatal plasma PTHrP value was similar to that of their mothers and to the mean plasma value of normal women of reproductive age. The lack of correlation between the paired fetal–maternal values that we observed implies that this peptide is probably not exchangeable between mother and fetus. Because of its low concentrations the specificity of the amino-terminal PTHrP(1–86) immunoreactivity that we found in plasma could not be validated further by examining the existence of parallelism of plasma dose–response lines to the standard curve of the assay or by chromatography: thus, the possibility that these PTHrP levels are non-specific “noise” of human plasma in the assay cannot be excluded. Currently, the prevailing opinion is that PTHrP plays no hormonal role in the adult human, where its conjectural functions are considered to be paracrine or autocrine (1, 2, 3, 10). Our observation that plasma amino-terminal PTHrP levels are similar among fetuses, newborn infants, pregnant mothers and normal controls casts some doubt upon the possible hormonal function of PTHrP(1–86) during fetal life or during pregnancy. Similar doubt is caused by the monotonous appearance of non-changing plasma levels of PTHrP during the second half of intrauterine life, a period of profound development and calcification of the fetal skeleton. In contrast, we found significantly higher plasma PTH in the fetuses at mid-gestation than in the newborns, although the meaning of this difference is not clear. The 1–34 regions of both PTHrP and PTH interact with the same conventional PTH receptor, and PTHrP...
and PTH usually have equimolar potency (18). From our data it seems that the total plasma PTH-like amino-terminal immunoreactivity is considerably lower during fetal compared to adult life, and in the fetus is due mainly to PTHrP(1–86). It should be noted, however, that the assays employed here do not detect small bioactive 1–34 fragments of either PTH or PTHrP. Our data indicate that the high levels of PTH bioactivity in umbilical cord blood at term reported previously, using a cytochemical bioassay (8, 9), are not due to intact PTH or PTHrP(1–86).

The placental calcium pump-stimulating activity of PTHrP initially reported by Rhodda et al. (7) was found subsequently to reside in the 75–85 region of the molecule by Care et al. (19). This prompted Malette (10) to hypothesize that a peptide containing the 75–85 region of PTHrP is the fetal calcemic hormone, with the placenta as one of its target tissues. Because the 1–34 region of PTHrP is important for fetal bone remodeling, the secreted PTHrP peptide would be expected to include at least the 1–84 region (10). In the present work we showed that such a peptide circulates at low concentration in human fetuses and newborns, confirming the similar findings of Thiébaud et al. (14). However, these investigators found levels of PTHrP(1–86) in cord blood of newborns at term to be significantly higher than in their mothers. A possible explanation for this discrepancy is that Thiébaud et al. (14) apparently used an earlier less-sensitive version of this commercial assay. We used a later version of the kit with enhanced sensitivity, which gives much lower values of PTHrP(1–86) in normal controls than previously reported with the older version of the assay (20) (P. Haaima, Nichols Institute of Diagnostics, pers. comm.). In fact, what Thiébaud et al. (14) found in their work was that the PTHrP(1–86) levels in cord blood at term were similar to the levels of normal non-pregnant women, while the pregnant mothers had PTHrP plasma levels lower than normal control non-pregnant women. Our findings agree with those of Thiébaud et al. (14) concerning the similarity of plasma PTHrP(1–86) levels between neonates and normal non-pregnant women. However, we did not find lower than normal control values in the corresponding pregnant mothers.

The actual source of the PTHrP in fetal plasma has not been established (10). One possibility is that the fetal parathyroids secrete PTHrP as a hormone (7) and the extracellular Ca\(^{2+}\)-sensing receptors are those of the parathyroid gland cells. However, the fetal parathyroid gland secretes PTH in response to induced hypocalcemia in primate fetuses in utero (21) or during spontaneous hypocalcemia occurring in preterm infants (9), therefore making the secretion of PTHrP by the human fetal parathyroid glands less likely.

Recently, Burtis et al. (22) showed that the most abundant circulating form of PTHrP in patients with humoral hypercalcemia of malignancy is a mid-region peptide beginning at residue 38, which has been shown to be a secretory form of PTHrP. Care et al. (19) found that three synthetic mid-region peptides—hPTHrP(75–84), hPTHrP(75–86) and the most active hPTHrP(67–86) amide—stimulated the sheep placental calcium pump. It is possible, therefore, that the form of PTHrP in the feto-placental circulation could be a mid-region peptide biologically active in the placenta that was not detected by any of the assays used in the previous and the present studies. Determination of the mid-region peptide of PTHrP in fetal and neonatal circulation could help to clarify this matter. The high level of the renally cleared (22) carboxy-terminal species of PTHrP found by Seki et al. (16) in neonatal serum is due probably to the slow clearance of this fragment from the fetal circulation (16). It is unlikely that the assay used by Seki et al. (16) detected a PTHrP peptide comprising both the mid- and carboxy-terminal regions, because such a peptide has not been identified to date (22). The carboxy-terminal PTHrP fragment has no known effect on calcium transport. On the other hand, the carboxy-terminal PTHrP peptide 107–111 region may inhibit osteoclastic bone resorption (10) and could play an anabolic role in the fetal skeleton.

The placenta itself is the most likely alternative source of fetal plasma PTHrP (10). The placenta is known to synthetize and secrete PTHrP (12, 23, 24). In addition, Juhlin et al. (25) showed that trophoblastic cells have calcium sensors similar to the Ca\(^{2+}\) receptors of parathyroid gland cells. Hellman et al. (12) demonstrated, by immunohistochemical staining, the presence of the amino-terminal and mid-regional part of the PTHrP molecule in the cytotrophoblasts. The same cells displayed surface staining with monoclonal antibodies, which recognize a Ca\(^{2+}\) receptor mechanism regulating hormone release of parathyroid cells. These investigators demonstrated that raised extracellular calcium inhibited release of PTHrP from the cytotrophoblasts, and this inhibition was blocked by the Ca\(^{2+}\) parathyroid gland cell receptor antibody. Thus, the placenta having both the calcium concentration sensing and PTHrP synthetizing and secreting capabilities could autoregulate its own calcium pump function in an autocrine or paracrine fashion. In this case, one might think that fetal plasma PTHrP has no hormonal function and represents the amount of peptide leaking to the plasma from the various fetal tissues in which it is produced locally.

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References


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