Parathyroid hormone-related protein (PTHrP) mRNA splicing and parathyroid hormone/PTHrP receptor mRNA expression in human placenta and fetal membranes

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ABSTRACT

During human pregnancy, parathyroid hormone-related protein (PTHrP) and parathyroid hormone (PTH)/PTHrP receptor are produced by the uterus, placenta, fetal membranes (amnion and chorion) and developing fetus. PTHrP alternative 3′ mRNA splicing results in transcripts which encode three PTHrP isoforms and have been identified in amnion. Uteroplacental PTHrP expression is greatest in amnion and increases dramatically during late pregnancy. The aims of this study were to determine PTH/PTHrP receptor mRNA expression at preterm and term gestations and to determine 3′ alternative splicing patterns in placenta, amnion and choriodecidua at preterm and term gestations. Using semiquantitative reverse transcription-polymerase chain reaction, PTHrP and PTH/PTHrP receptor transcripts were identified in preterm (n=5) and term (n=7) gestational tissues. PTH/PTHrP receptor mRNA expression did not differ between tissue types or change with advancing gestation. In contrast, PTHrP expression in the same tissues increased with advancing gestation and was significantly greater in amnion than in placenta and choriodecidua. Thus PTHrP, although produced predominantly in amnion, may act in amnion and other tissues including placenta, choriodecidua and myometrium.

In amnion over placenta, transcripts encoding PTHrP 1-139 and 1-173 were detected in some preterm and all term samples and those encoding PTHrP 1-141 were detected in all samples. Similar results were obtained for reflected amnion. In placenta and choriodecidua, PTHrP 1-139 and 1-173 transcripts were undetectable or of low abundance. PTHrP 1-141 transcripts were detected in some placenta and choriodecidua samples.

In summary, transcripts encoding PTHrP 1-141 appeared to be more abundantly expressed than those encoding PTHrP 1-139 or 1-173. However, the up-regulation of PTHrP expression in amnion at term may involve each of the alternative 3′ mRNA splicing pathways since transcripts for each isoform appeared to be more consistently expressed at term.
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INTRODUCTION

Parathyroid hormone-related protein (PTHrP) is a tumour product and a causative agent in the hypercalcaemic syndrome associated with several cancers; however, the specific expression of PTHrP in many non-malignant tissues indicates important roles in normal physiology (Moseley & Gillespie 1995, Philbrick *et al*. 1996). The PTHrP gene is complex with nine exons and three distinct promoter regions (Mangin *et al*. 1989, 1990, Suva *et al*. 1989, Vasavada *et al*. 1993). Only two exons are present in all PTHrP transcripts, exon V which encodes the prepro region of PTHrP and exon VI which encodes most of the mature protein (Moseley & Gillespie 1995). Alternative 3′ splicing to exons VII, VIII and IX produces transcripts encoding three different isoforms of the protein, PTHrP

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1-139, 1-173 and 1-141 respectively (Mangin *et al*. 1988, Thiede *et al*. 1988, Yasuda *et al*. 1989). Alternative 3′ splicing has been demonstrated in several human cell lines and tissues and splicing events may be tissue-specific and regulated by hormones, growth factors and cytokines (Brandt *et al*. 1992, Campos *et al*. 1994, Southby *et al*. 1995, 1996). PTHrP exhibits N-terminal homology with parathyroid hormone (PTH) which allows the two proteins to bind to a common PTH/PTHrP receptor (Juppner *et al*. 1991, Abou-Samra *et al*. 1992). The PTH/PTHrP receptor is a seven transmembrane domain, G-protein-linked receptor which signals via both adenylate cyclase and phospholipase C (Juppner *et al*. 1991, Abou-Samra *et al*. 1992, Schipani *et al*. 1993).

During pregnancy PTHrP is produced in the uterus, placenta, and many fetal tissues and is thought to act locally in an autocrine/paracrine manner. Although the specific roles of PTHrP in normal fetal development are not clearly defined, both PTHrP and its receptor have been identified in fetal skin, epithelia including lung and gut epithelia and developing bone (Campos *et al*. 1991, Moseley *et al*. 1991, Senior *et al*. 1991, Karperien *et al*. 1994, Lee *et al*. 1995). PTHrP-deficient mice, generated by partial PTHrP gene deletion and homologous recombination, die at birth and exhibit severe skeletal dysplasia (Karaplis *et al*. 1994). PTHrP expression has been identified in uterine tissues and may be regulated by myometrial stretch (Thiede *et al*. 1990, Beck *et al*. 1993) and autocrine and paracrine mediators (Thiede *et al*. 1991, Casey *et al*. 1992). PTHrP inhibits contraction of rat myometrium *in vitro* (Shew *et al*. 1991, Dalle *et al*. 1992, Paspaliaris *et al*. 1992, Williams *et al*. 1994) and may help to maintain uterine relaxation during pregnancy. PTHrP mRNA has been identified in human myometrium at term (Ferguson *et al*. 1992); however, PTH/PTHrP receptor expression in human myometrium has not been studied in detail.

PTHrP mRNA and protein have also been identified in human gestational tissues including placenta, amnion, chorion and decidua (Ferguson *et al*. 1992, Germain *et al*. 1992, Bowden *et al*. 1994, Emly *et al*. 1994, Bruns *et al*. 1995, Curtis *et al*. 1997). PTHrP has been shown to stimulate ovine placental calcium transfer from maternal to fetal circulations (Care *et al*. 1990), although this has not yet been demonstrated in humans. PTHrP also stimulates vasodilatation of the fetal-placental circulation (Mandsager *et al*. 1994, Macgill *et al*. 1997). In human gestational tissues collected at term deliveries, the expression of PTHrP mRNA and immunoreactive protein was greater in amnion than in placenta and choriodecidua and was greater in amnion over placenta than in reflected amnion (Ferguson *et al*. 1992, Germain *et al*. 1992, Curtis *et al*. 1997). Thus, PTHrP expression in human gestational tissues appeared tissue-specific at term. We recently detected PTHrP mRNA and protein in these tissues at preterm gestations and demonstrated that both increased dramatically with advancing gestation in the fetal membranes, particularly in amnion (Curtis *et al*. 1997). PTHrP concentrations in human amniotic fluid also increased between preterm and term (Wlodek *et al*. 1995). The apparent up-regulation of PTHrP expression in amnion and accumulation of PTHrP in amniotic fluid at term suggest that amnion-derived PTHrP is important during late pregnancy. PTH/PTHrP receptor mRNA has previously been identified by reverse transcription-polymerase chain reaction (RT-PCR) in term placenta, umbilical cord, amnion and chorion-decidua (Bruns *et al*. 1995); however, no studies to date have examined receptor expression in these tissues at both preterm and term gestations.

In this study, PTH/PTHrP receptor transcripts have been identified for the first time in human gestational tissues at both preterm and term. We hypothesised that PTH/PTHrP receptor mRNA expression was tissue-specific and inversely correlated with PTHrP expression, thus the relative abundance of PTH/PTHrP receptor mRNA was determined by semiquantitative RT-PCR in order to examine the relationship to PTHrP mRNA expression in the same preterm and term tissues. Brandt *et al*. (1992) detected PTHrP transcripts for all three isoforms of PTHrP in two samples of amnion and reported that the predominant 3′ splicing products in amnion specified PTHrP 1-139. However, PTHrP mRNA 3′ splicing patterns in amnion, chorion and placenta have yet to be fully investigated. Therefore, to determine whether PTHrP mRNA 3′ splicing is tissue-specific and which transcripts are present at preterm and term, we have utilised RT-PCR with PTHrP exon-specific primers. Finally, we have used RT-PCR with PTHrP common exon (V and VI) primers to detect low abundance PTHrP mRNA in tissues where it was previously undetectable by Northern blot analysis.

MATERIALS AND METHODS

Materials

(α-^{32}P)ATP (3000 Ci/mmol) and Hybond-N membrane were obtained from Amersham International (Amersham, Bucks, UK). Ultrapure dNTP Set was purchased from Amrad Pharmacia Biotech

TABLE 1. PCR primer combinations and the respective transcripts amplified, isoforms encoded and exonic sequences contained, predicted PCR product sizes in base pairs and optimal PCR amplification cycle number

	Transcripts	Exons	Product size	Optimal cycles
Primer pairs				
obrf 15.84–obrf 15.89	all PTHrP transcripts	V–VI	161	28
obrf 15.84–obrf 15.90	139 aa isoforms	V–VI–VII	603	—
obrf 15.84–obrf 15.91	173 aa isoforms	V–VI–VIII	565	—
obrf 15.84–obrf 15.92	141 aa isoforms	V–VI–IX	543	—
obrf 15.112–obrf 15.113	PTH/PTHrP receptor	—	449	34
GAPDH-3–GAPDH-4	GAPDH	5–6–8	414	26
18.01–18.02	18S ribosomal RNA	—	597	24

aa, amino acids.

(North Ryde, NSW, Australia). DNA molecular weight marker (VI) was obtained from Boehringer Mannheim (Mannheim, Germany). Guanidinium thiocyanate was purchased from Fluka (Buchs, Switzerland). Amplitaq DNA polymerase was purchased from Perkin Elmer (Branchburg, NJ, USA). Molecular biology grade agarose, avian myeloblastosis virus reverse transcriptase, oligo $(dT)_{15}$ primer, random hexamer primer and T4 polynucleotide kinase were obtained from Promega (Madison, WI, USA). Ficoll and polyvinylpyrrolidone (PVP) were purchased from Sigma (St Louis, MO, USA). Phenol was purchased from Wako (Osaka, Japan). All other reagents were of analytical grade and were purchased from standard suppliers.

Patients and samples

This project was approved by the Royal Women's Hospital Research and Ethics Committees and written informed consent was obtained from participating patients. Placentae and fetal membranes were collected at delivery from women with spontaneous-onset labour and normal vaginal delivery at term gestation (37–42 weeks, n=7) and from women with idiopathic preterm labour and spontaneous vaginal delivery at preterm gestation (<36 weeks, n=5). Myometrium was obtained at elective caesarean section from one non-labouring, term pregnant woman. All patients were otherwise healthy women with normal singleton pregnancies. Tissue processing was commenced within 15 min of delivery of the placenta. Placental villous tissue (primarily trophoblast cells) was dissected from the maternal surface or basal plate. Amnion membrane overlying the fetal surface of the placenta or chorionic plate (amnion over placenta) and amnion membrane overlying chorion laeve (reflected amnion) peeled readily from the underlying tissue and thus were obtained by blunt dissection. A sample of chorion laeve with adherent decidual cells (choriodecidua) was also obtained. Total RNA was extracted immediately following dissection using a guanidinium thiocyanate-phenol-chloroform extraction procedure (Chomczynski & Sacchi 1987). Total RNA was also extracted from MDA-MB-231 cells which express PTHrP and PTH/PTHrP receptor mRNA (Southby *et al*. 1996) as a positive control.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA (5·0 μg) was reverse transcribed with 0·5 μg oligo (dT) primer, 10 mM dithiothreitol, 0·25 mM each of dATP, dCTP, dGTP and dTTP and 15 units avian myeloblastosis virus reverse transcriptase in a final reaction volume of 25 μl in 1 × AMV-RT buffer (Promega). The reaction was incubated at 42 °C for 2 h. In order to allow PCR amplification of 18S ribosomal RNA, each sample was also reverse transcribed using random hexamer primers. One twenty-fifth of the total reaction was used in a PCR with 25 pmol each of the forward and reverse primers, 25 μM each of dATP, dCTP, dGTP and dTTP, and 1 unit Amplitaq DNA Polymerase in a final volume of 20 μl in 1 × Amplitaq buffer (Perkin Elmer). The reactions were overlaid with 50 μl paraffin oil and exposed to thermal cycling at 95 °C for 30 s (denaturation), 55 °C for 30 s (primer annealling) and 72 °C for 60 s (extension) in an FTS-320 Thermal Sequencer (Corbett Research, Sydney, NSW, Australia). Forty amplification cycles were used for all PCR reactions, except for semiquantitative RT-PCR analyses in which the optimal sub-saturation cycle number was determined for each primer pair, as previously described (Southby *et al*. 1996). All oligonucleotide primers were synthesised on an Oligo 1000M DNA Synthesizer (Beckman Instruments, Fullerton, CA, USA). The PCR primer combinations are described in Table 1. The PTHrP primers were obrf 15.84

(sense strand-specific) and obrf 15.89 (antisense strand-specific), directed at exons V and VI respectively, which are exons common to all PTHrP transcripts. The PTHrP 3′ exon-specific antisense primers, which were used in combination with obrf 15.84 to detect alternative splicing to each of the 3′ ultimate exons, were obrf 15.90 (exon VII), obrf 15.91 (exon VIII) and obrf 15.92 (exon IX). The sequence, orientation and position of the PTHrP, PTH/PTHrP receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) PCR primers and the respective detection oligonucleotides have been described previously (Southby *et al*. 1995). The human 18S ribosomal RNA primers were 18.01 (5′-TTGTTGGTTTTCGGAACTG AGG-3′, sense strand-specific, nucleotides 889-910) and 18.02 (3′-GTCGGTGGGCTCTAACTCG TTA-5′, antisense strand-specific, nucleotides 1485-1464) for amplification and an internal sense strand oligonucleotide for detection, 18.03 (5′-AGGAAT TGACGGAAGGGCAC-3′, nucleotides 1196-1215). PCR negative controls were no-template reactions and reactions using RNA that had not been reverse transcribed. PCR products were not detected in these controls and the PCR conditions were stringent as non-specific amplification products were not detected. Each PCR was performed in duplicate or triplicate and the results were reproducible.

PCR products were resolved on 2% agarose/1 × Tris-Borate-EDTA gels. DNA molecular weight markers were included on each gel in order to confirm PCR product sizes. To facilitate transfer, the gels were soaked sequentially in 0·25 M HCl for 20 min, 0·5 M NaOH/1·5 M NaCl for 30 min and 1·0 M Tris–HCl/1·5 M NaCl for 20 min (Maniatis *et al*. 1982). The amplified products were transferred to Hybond-N membrane by capillary blotting with 20 × SSC and were immobilised by UV cross-linking. Specific internal oligonucleotides (50 pmol) were 5′ end-labelled with (α-^{32}P)ATP and polynucleotide kinase (Maniatis *et al*. 1982) and were used to confirm the specificity of the PCR. Membranes were incubated for 1-2 h at 42 °C in hybridisation buffer (15% formamide, 5 × Denhardt's solution (0·1% BSA, 0·1% Ficoll, 0·1% PVP), 6 × SSPE, 0·025% skimmed milk powder and 0·1% SDS) and then hybridised with the radiolabelled probe overnight at 42 °C. The membranes were washed to a stringency of 1 × SSC/0·1% SDS at 42 °C for 10 min. Hybridisation signals were detected and quantitated using PhosphorImager analysis and laser densitometry. Both GAPDH mRNA and 18S ribosomal RNA were detected for normalisation; however, GAPDH was not used in semiquantitative analyses due to recent evidence that its expression varies significantly between human gestational tissue types (Freed *et al*. 1997). Thus, for semiquantitative analyses, the intensity of hybridisation for a given amplification product was expressed as a ratio of the positive control on the filter and was normalised against the respective 18S ribosomal RNA signal. As 18S ribosomal RNA PCR was performed on random-primed rather than oligo (dT)-primed RT reactions, these methods were compared by performing PTHrP and GAPDH PCR on several samples primed by either method. The resultant amplicons were similarly abundant for the two types of reverse transcription primer used.

Statistical analysis

The data were analysed for homogeneity of variance using Bartlett's test and were found to be non-homogeneous. The data, therefore, were log-transformed for statistical analysis. Significant differences were detected using one-way analysis of variance (ANOVA) and group means were compared using multiple range tests (Student Newman-Keuls). $P<0·05$ was considered significant.

RESULTS

Expression of PTHrP and PTH/PTHrP receptor at preterm and term

Using RT-PCR, PTHrP and PTH/PTHrP receptor mRNA expression were detected in human gestational tissues at preterm and term. PCR with the PTHrP common exon (V-VI) primers and with PTH/PTHrP receptor primers was initially performed under saturating conditions in order to ensure detection of very low abundance transcripts, thus samples underwent 40 cycles of PCR amplification. Gestational tissues from 3 preterm and 3 term deliveries were analysed. In each sample a band was observed which corresponded to the predicted PCR product size for the target sequence and which subsequently hybridised with a specific internal oligonucleotide probe (Fig. 1A). GAPDH and 18S ribosomal RNA sequences were detected by PCR on the same RT reactions to confirm the integrity of the total RNA samples and the RT activity. Both PTHrP and PTH/PTHrP receptor mRNA were detected in all the preterm and term tissues examined, including placenta, amnion over placenta, reflected amnion, choriodecidua and one term myometrium sample.

RT-PCR analysis of PTHrP and PTH/PTHrP receptor expression was also performed under semiquantitative conditions using the optimal number of PCR cycles (Table 1). PTHrP and

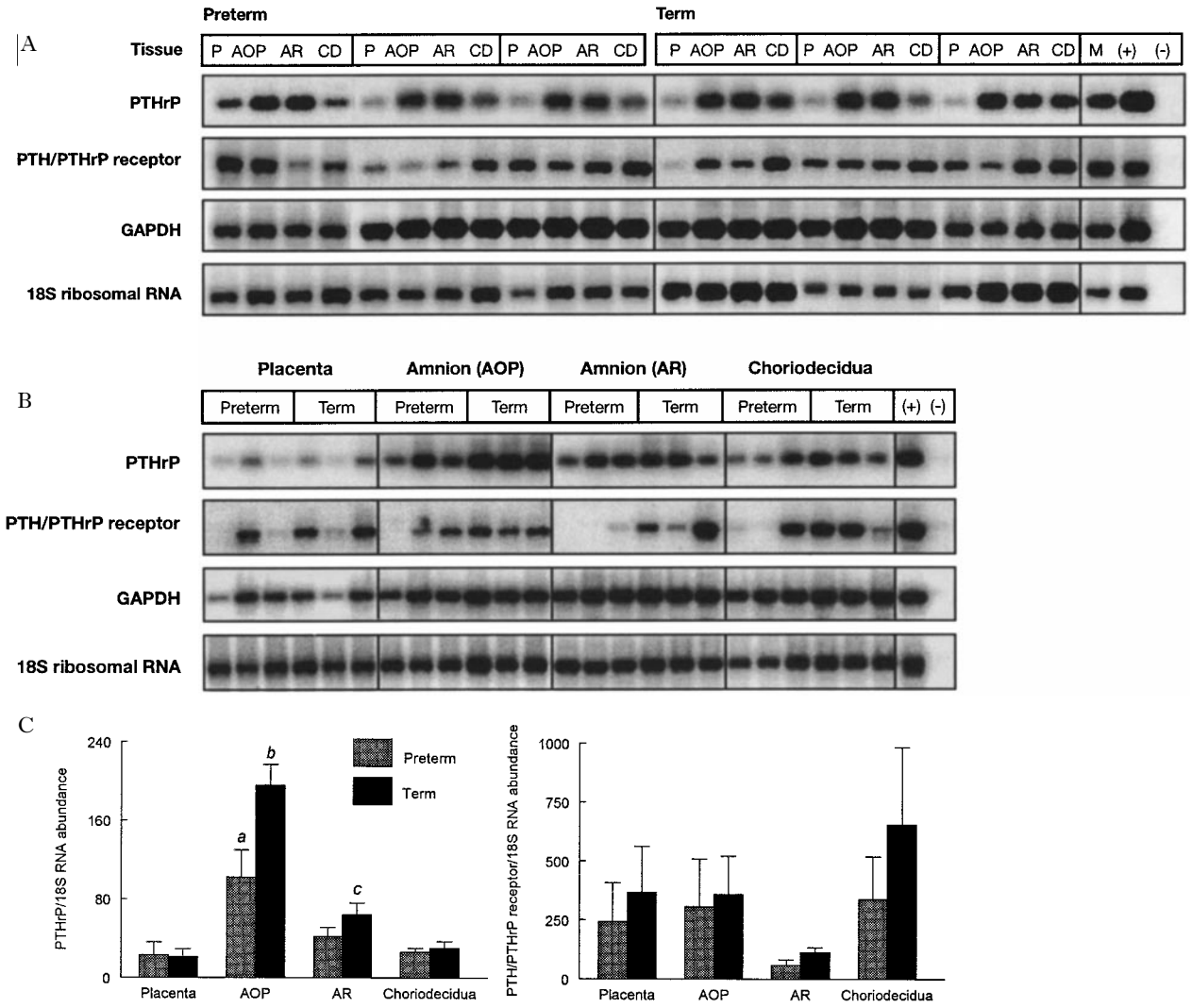

FIGURE 1. RT-PCR detection of PTHrP mRNA, PTH/PTHrP receptor mRNA, GAPDH mRNA and 18S ribosomal RNA sequences in human placenta (P), amnion over placenta (AOP), reflected amnion (AR) and choriodecidua (CD) from 3 preterm and 3 term deliveries, in term myometrium (M), a positive (+) control (MDA-MB-231 cells) and a negative (−) control (no template PCR). (A) Target sequence cDNAs were amplified under saturating conditions using 40 cycles of PCR with specific primers and (B) PTHrP, PTH/PTHrP receptor, GAPDH and 18S ribosomal RNA cDNAs were amplified under semiquantitative conditions using 28, 34, 26, and 24 cycles of PCR respectively. PCR products were resolved on 2% (w/v) agarose gels, transferred to nylon filters and hybridised with radiolabelled internal oligonucleotides. Autoradiographs were obtained by exposure of the filters to Phosphorimager screens for 2-16 h. For the semiquantitative RT-PCR analysis of each target sequence, all 24 samples and the controls were resolved on a single agarose gel in order to minimise error. (C) The relative expression of PTHrP mRNA and PTH/PTHrP receptor mRNA as determined by semiquantitative RT-PCR in placenta, amnion over placenta (AOP), reflected amnion (AR) and choriodecidua collected at preterm (n=5 for each tissue except placenta n=4) and term (n=7 for each tissue except placenta n=5). Hybridisation signals were quantitated by Phosphorimaging and normalised against the corresponding 18S ribosomal RNA signals. The data are presented as means ± S.E.M. The relative expression of PTHrP was significantly elevated in preterm amnion over placenta compared with all other preterm tissues (*a*, P<0·05). The relative expression of PTHrP was significantly elevated at term compared with preterm in amnion over placenta and was elevated compared with all other term tissues (*b*, P<0·05). The relative expression of PTHrP was significantly elevated in term reflected amnion compared with term placenta and choriodecidua (*c*, P<0·05).

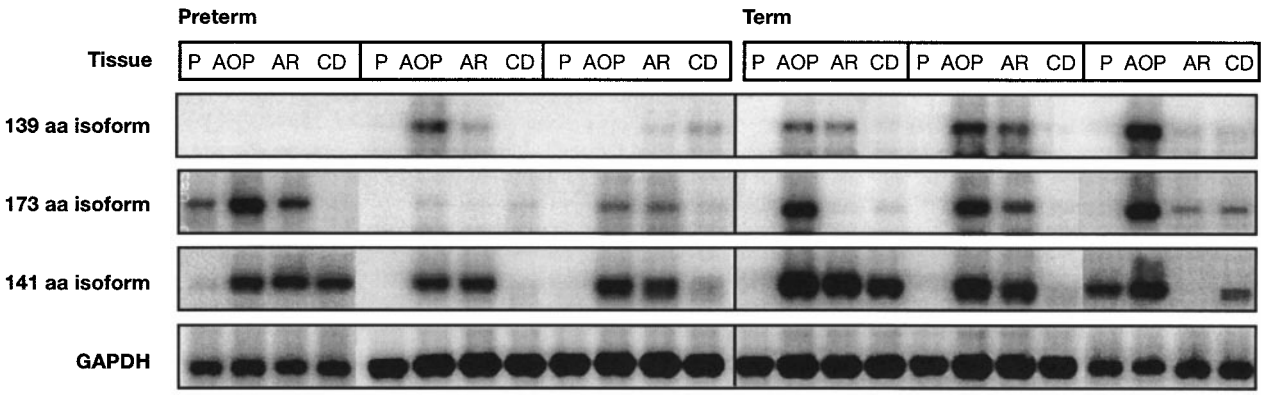

FIGURE 2. RT-PCR analysis of PTHrP mRNA 3′ splicing patterns in placenta (P), amnion over placenta (AOP), reflected amnion (AR) and choriodecidua (CD) from 3 preterm and 3 term deliveries. Forty cycles of PCR were performed using a sense strand-specific primer to PTHrP exon V with antisense strand-specific primers to alternative 3′ exons VII, VIII and IX to detect transcripts encoding the 139, 173 and 141 amino acid (aa) isoforms of PTHrP respectively. GAPDH sequences were amplified using 40 cycles of PCR with specific primers. PCR products were resolved on 2% (w/v) agarose gels, transferred to nylon filters and hybridised with radiolabelled internal oligonucleotides. Autoradiographs were obtained by exposure of the filters to Phosphorimager screens for 2-16 h.

PTH/PTHrP receptor transcripts were detected in most tissues following 28 and 34 cycles of PCR respectively (Fig. 1B). Three of the twelve placenta samples were excluded from analyses due to poor integrity (GAPDH and 18S ribosomal RNA sequences not amplifiable by PCR). PTHrP transcripts were detected in low abundance in all of the placenta and choriodecidua samples. PTH/PTHrP receptor mRNA expression was highly variable between patients; receptor transcripts were clearly detected in 5 of 9 placenta and 9 of 12 choriodecidua samples but were almost undetectable in the remaining samples. PTHrP transcripts were clearly detected in all samples of amnion over placenta (n=12) and reflected amnion (n=12) and PTH/PTHrP receptor mRNA expression in these tissues was variable. The data were normalised and the relative expression of PTHrP and receptor transcripts was compared between tissue types and gestations (Fig. 1C). Significant differences in PTHrP expression were detected between tissue types and between preterm and term gestations (ANOVA, P<0·05). Preterm PTHrP mRNA expression was significantly elevated in amnion over placenta compared with other tissues (a, P<0·05). Likewise, term PTHrP mRNA expression was significantly greater in amnion over placenta (b, P<0·05). Term PTHrP mRNA expression was also greater in reflected amnion than in placenta or choriodecidua (c, P<0·05). PTHrP expression increased significantly between preterm and term in amnion over placenta (b, P<0·05). The relative expression of PTH/PTHrP receptor mRNA was not significantly different between tissues and did not appear to change between preterm and term gestations. No correlations were observed between PTH/PTHrP receptor mRNA expression and PTHrP mRNA expression.

PTHrP mRNA alternative 3′ splicing at preterm and term

In order to determine which isoforms of PTHrP are produced in placenta, amnion over placenta, reflected amnion and choriodecidua and whether this differs between preterm and term, alternative 3′ splicing events were examined by RT-PCR (40 cycles) with a sense strand-specific primer to PTHrP common exon V and antisense strand-specific primers to alternative 3′ exons VII, VIII and IX. In these experiments, the samples were reverse transcribed RNA from tissues collected at 3 preterm and 3 term deliveries (the same samples as represented in Fig. 1A). Bands were observed which corresponded to the predicted PCR product sizes for the target sequences and which subsequently hybridised with specific internal oligonucleotide probes (Fig. 2). In placenta and choriodecidua, transcripts containing exon VII (encoding PTHrP 1-139) were undetectable and transcripts containing exon VIII (encoding PTHrP 1-173) were detected in only 1 of 6 samples. Those transcripts containing exon IX (encoding PTHrP 1-141) were detected in 1 term placenta sample and in 2 of 3 preterm and 2 of 3 term choriodecidua samples (Fig. 2). In amnion over placenta, transcripts encoding PTHrP 1-139

were detected in 1 of 3 preterm and all term samples, those encoding PTHrP 1-173 were detected in 1 of 3 preterm and all term samples, and those encoding PTHrP 1-141 were detected in all preterm and term samples. Similar results were obtained for reflected amnion. In amnion over placenta and reflected amnion, all 3 types of PTHrP transcript appeared to be more consistently or abundantly expressed at term than at preterm. Also, the transcripts encoding PTHrP 1-141 appeared more abundant than those encoding PTHrP 1-139 or 1-173 in preterm and term amnion samples.

DISCUSSION

In this study, RT-PCR was utilised to identify potential target tissues for PTHrP within the uteroplacental unit and to examine the relationship between PTHrP expression and PTH/PTHrP receptor expression at preterm and term gestations. PTHrP and PTH/PTHrP receptor transcripts were clearly detected in myometrium and in preterm and term samples of human placenta, amnion over placenta, reflected amnion and choriodecidua by RT-PCR under saturating conditions. Thus, PTHrP potentially acts in each of these gestational tissue types. This is the first time PTH/PTHrP receptor mRNA expression has been identified in preterm human uteroplacental tissues. Interestingly, we clearly detected PTH/PTHrP receptor mRNA in every amnion sample which is in contrast to previous findings. Bruns *et al*. (1995) utilised RT-PCR to detect PTH/PTHrP receptor mRNA in human fetal membranes from five term deliveries and detected only weak or absent signals for receptor transcripts in amnion over placenta and reflected amnion. However, this discrepancy may be explained by methodological differences since in the present study 5·0 μg total RNA together with 34 or 40 PCR cycles were used compared with 1·0 μg total RNA and 25 PCR cycles used in the previous study (Bruns *et al*. 1995). We also clearly detected PTHrP mRNA in all of the preterm and term tissues examined. This confirmed the presence of PTHrP mRNA in placenta and choriodecidua, particularly in preterm tissues in which we had previously found it undetectable by Northern blot analysis (Curtis *et al*. 1997), demonstrating the value of using the more sensitive RT-PCR technique for detecting low abundance transcripts.

The analysis was repeated on the same samples under semiquantitative PCR conditions to allow some comparison of the relative abundance of PTHrP and receptor transcripts between tissue types and gestations. The profile of PTHrP expression was similar to that which we observed by Northern blot analysis and to protein levels determined by radioimmunoassay (Curtis *et al*. 1997). The relative expression of PTH/PTHrP receptor mRNA in the same tissues was not different between tissue types or gestations. While PTH/PTHrP receptor mRNA was detected in each type of tissue, expression was variable between patients. There was no clear pattern of expression and no correlation with PTHrP mRNA expression. Thus, PTH/PTHrP receptor mRNA expression is not tissue-specific and is not down-regulated in amnion at term in association with increased PTHrP expression. The regulation of PTH/PTHrP receptor expression is not understood, therefore, it is difficult to speculate why PTH/PTHrP receptor expression appeared to be variable in uteroplacental tissues. However, if unique (non-PTH) receptors for PTHrP exist in uteroplacental tissues, as has been suggested (Moseley & Gillespie 1995), the PTH/PTHrP receptor could be redundant in some of these tissues. Bruns *et al*. (1995) identified PTH/PTHrP receptor mRNA in human gestational tissues at term and suggested it was abundant in placenta and chorion-decidua but low or undetectable in amnion. This suggested that amnion-derived PTHrP may act in a paracrine manner in adjacent placenta or choriodecidua. In contrast, we have detected PTH/PTHrP receptor mRNA in all gestational tissue types, including amnion, and did not find a difference in abundance between tissues, suggesting that amnion-derived PTHrP may also act in an autocrine manner.

We have previously demonstrated that both PTHrP mRNA expression and immunoreactive PTHrP increase in the human fetal membranes, amnion and chorion in the final 6-8 weeks of pregnancy (Curtis *et al*. 1997) and that PTHrP accumulates in human amniotic fluid, with concentrations increasing significantly with advancing gestation (Wlodek *et al*. 1995). Amnion is a likely source of the high concentrations of PTHrP in amniotic fluid and PTHrP is known to have a role in normal fetal development (Karaplis *et al*. 1994). Thus, although PTHrP is produced locally in fetal tissues (Campos *et al*. 1991, Moseley *et al*. 1991, Senior *et al*. 1991), amnion-derived PTHrP in human amniotic fluid could also affect the fetus. Amnion-derived PTHrP may also stimulate growth and differentiation of amnion epithelium. In this study, PTH/PTHrP receptor transcripts were identifed in preterm and term uteroplacental tissues suggesting that PTHrP can act in placenta, choriodecidua, myometrium and amnion. Potential physiological functions of PTHrP during pregnancy include stimulation of placental calcium transport

from mother to fetus (Care *et al*. 1990), vasodilatation of the fetal-placental vasculature (Macgill *et al*. 1997), relaxation of uterine smooth muscle (Williams *et al*. 1994) and cell growth and differentiation (Moseley *et al*. 1991).

RT-PCR has been utilised in several previous studies to examine PTHrP alternative 3′ splicing in various cell lines and tissues and it has been suggested that splicing of PTHrP transcripts may be tissue-specific (Brandt *et al*. 1992, Campos *et al*. 1994, Southby *et al*. 1995, 1996). Brandt *et al*. (1992) demonstrated that splicing to each of the alternative 3′ exons must occur in human amnion, as transcripts containing exons VI, VIII and IX were detected by PCR with exon-specific primers. Human amnion, therefore, may potentially express the three isoforms of PTHrP which are 139, 173 and 141 amino acids in length. Brandt *et al*. (1992) also suggested that splicing to produce transcripts encoding PTHrP 1-139, rather than PTHrP 1-173 or 1-141, was preferential in human amnion. Studies of PTHrP alternative 3′ splicing in other normal tissues, tumours and human cell lines suggested that PTHrP 1-141 was the most ubiquitously expressed isoform (Nakamura *et al*. 1995, Southby *et al*. 1995). Thus, PTHrP mRNA 3′ splicing patterns required further investigation in amnion and had not been previously reported for placenta or choriodecidua or for any preterm tissues. We utilised RT-PCR with PTHrP exon-specific primers to determine whether PTHrP mRNA 3′ splicing was tissue-specific in human placenta, amnion and choriodecidua and which transcripts were present at preterm and term. Different patterns of alternative 3′ splicing were not apparent between tissue types. Indeed splicing to each of the alternative 3′ exons may occur in human placenta, amnion and choriodecidua since PTHrP transcripts containing exons VII, VIII and IX were detected. Transcripts encoding PTHrP 1-141, however, appeared to be more abundant than those encoding PTHrP 1-139 or 1-173 in amnion and choriodecidua. PTHrP transcripts encoding each isoform were more consistently or more abundantly expressed at term than at preterm, suggesting that all of the 3′ mRNA splicing pathways may be involved in the up-regulation of PTHrP expression in human fetal membranes at term gestation. It is unlikely that the three predicted C-terminal sequences mediate different biological actions, since between species, the PTHrP sequence is divergent beyond amino acid 111 (Moseley & Gillespie 1995). The physiological significance of the expression of three PTHrP mRNA isoforms in human gestational tissues probably relates to the opportunity for diversity in translational regulation and post-translational processing.

PTHrP expression appears to be regulated in a tissue-specific manner within the human utero-placental unit and is upregulated in amnion in late gestation. The data in this study indicated that all three isoforms of PTHrP may be produced in placenta, amnion and choriodecidua at both preterm and term gestations. Receptor expression appeared not to be as strongly regulated as PTHrP expression since it did not differ between tissue types or gestations. The presence of receptor transcripts, however, suggested that PTHrP may have autocrine and paracrine functions in all preterm and term tissues, including amnion, choriodecidua, placenta and myometrium.

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