Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6969–6973, September 1990
Medical Sciences

Intrauterine occupancy controls expression of the parathyroid hormone-related peptide gene in preterm rat myometrium

(gene family/gravid uterus/parturition)

MARK A. THIEDE*, ANASTASIA G. DAIFOTIS†, ELEANOR C. WEIR‡, MICHAEL L. BRINES§, WILLIAM J. BURTIS¶, KYOJI IKEDA†, BARBARA E. DREYER†, ROBERT E. GARFIELD‖, AND ARTHUR E. BROADUS†**

*Department of Bone Biology and Osteoporosis Research, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486; †Division of Endocrinology, ‡Section of Comparative Medicine, and §Neuroendocrine Program, Yale University, New Haven, CT 06510; ¶Division of Endocrinology, West Haven Veterans Administration Medical Center, West Haven, CT 06516; and ‖Departments of Biomedical Sciences and Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada L8N 3Z5

Communicated by Aaron B. Lerner, June 21, 1990

ABSTRACT **The parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHRP) genes are members of a gene family. Whereas PTH is a classical peptide hormone, mounting evidence suggests that the PTHRP may have predominately local actions. We report here that the PTHRP gene is expressed in rat myometrium, with a major peak in PTHRP mRNA expression occurring in the 48 hr immediately preceding parturition. A similar peak in peptide content was found in tissue extracts by biological and immunological assays, but the PTHRP could not be detected in the peripheral circulation or in uterine vein plasma during late gestation. By *in situ* hybridization histochemistry, PTHRP mRNA was demonstrated in both the longitudinal and circular layers of smooth muscle but was absent in the endometrium. The rise in myometrial PTHRP mRNA in late gestation was dependent upon intrauterine occupancy; it was greatly reduced or absent in nongravid uterine horns. These findings indicate that the expression of the PTHRP gene in preterm myometrium is under the control of a local stimulus and suggest that the PTHRP may play a paracrine or autocrine role in the uterus during the antepartum period, possibly involving myometrial contractility.**

The parathyroid hormone-related peptide (PTHRP) was initially isolated (1–4) and its cDNA cloned (5–8) from human and animal tumors associated with the syndrome of humoral hypercalcemia of malignancy. The PTHRP has sequence similarity to parathyroid hormone (PTH) at its N terminus, which appears to account for its PTH-like actions when it is secreted into the systemic circulation by malignant tumors (9). However, the deduced PTHRP product is larger than PTH and has a more complex structure, suggesting that it may be subject to one or several posttranslational processing steps (5–8). The precise secretory form of the PTHRP has not been established for any cell type.

Chromosomal localization studies and the structures of the PTH and PTHRP genes indicate that they arose by duplication from a common ancestral chromosome (10–12). Following this duplication event, the two genes have clearly evolved separately. The human PTHRP gene has been found to have an organization that is considerably more complex than that of the PTH gene (10–14). In addition, whereas the PTH gene is expressed predominately if not exclusively in parathyroid cells (15), the PTHRP gene is widely expressed in both endocrine and nonendocrine tissues. These sites of expression include tissues as diverse as skin (6, 16, 17), lactating mammary tissue (8), the chicken oviduct shell gland (18), the endocrine pancreas (19), and regions of the central nervous system (20). The role(s) of the PTHRP in these various sites is unknown, but the available evidence suggests that the peptide may be acting locally. Unique PTHRP receptors have not yet been identified.

We report here that the PTHRP gene is expressed in rat myometrium and that this expression peaks in the antepartum period. This peak in PTHRP gene expression is confined to gravid uterine horns, suggesting that it is under the control of a local stimulus, possibly derived from the fetoplacental unit.

METHODS

Animals. Timed-pregnant Sprague–Dawley rats were obtained from Taconic Farms, with day 1 of gestation being defined by the time of identification of the cervical plug. Following sacrifice, uterine tissues were snap frozen and stored at −70°C. In one group of animals, parturition was prevented by administering progesterone (5 mg in oil intramuscularly daily) beginning on day 19 (21). Gestation continued in these animals to day 24, some 36–48 hr after parturition would have normally occurred (21).

Surgical procedures were carried out under methoxyflurane anesthesia. Bilateral oophorectomy (or a sham operation) was performed on the afternoon of day 19, and the animals were sacrificed on the morning of day 21. Unilateral pregnancies were produced by performing a unilateral tubal ligation 24 hr after identification of the cervical plug, before the egg had traversed the oviduct to reach the uterus.

RNA Blot and RNase Protection Analyses. Total cellular RNA was prepared by a modification of the guanidinium isothiocyanate/cesium chloride technique (17). For blot analysis, RNA (30 μg) was separated on a 1% agarose/formaldehyde gel, transferred to nitrocellulose, and probed with a 712-base-pair *Pvu* II/*Xba* I fragment containing the coding region and 3′ untranslated region of the rat PTHRP cDNA (8). Blots were subsequently reprobed with a 770-base-pair *Nco* I/*Taq* I fragment of the chicken β-actin cDNA (22). The probes were labeled by the random primer technique (23). RNase protection analysis was carried out by using 30 μg of total RNA and 2×10^5 cpm of an antisense probe corresponding to the coding region of the rat PTHRP cDNA (20, 24). A 343-base-pair *Pvu* II/*Bgl* II fragment of this cDNA was subcloned into Bluescript vector (Stratagene), and ^{32}P-labeled RNA was prepared by using T7 RNA polymerase (17). Pro-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PTH, parathyroid hormone; bPTH, bovine PTH; PTHRP, PTH-related peptide; hPTHRP, human PTHRP; ROS, rat osteosarcoma; IRMA, immunoradiometric assay.

**To whom reprint requests should be addressed at: Fitkin I, Department of Internal Medicine, Yale University, 333 Cedar Street, New Haven, CT 06510.

tected fragments were fractionated on a 5% polyacrylamide/7 M urea gel.

Tissue Extraction and Biological and Immunological Assays. Acid/urea extracts of uteri were prepared as described (25) and examined for PTHRP biological activity using the PTH-sensitive rat osteosarcoma (ROS) cell assay, which measures the conversion of [^{3}H]ATP to cyclic [^{3}H]AMP (26). The standard in the assay was synthetic human PTHRP [hPTHRP-(1–74)] (27); the human and rat PTHRPs differ by only one residue through the first 74 amino acids (5–8). Specificity of binding was demonstrated by using the synthetic bovine PTH (bPTH) competitive inhibitors [Nle^{8,18}, Tyr^{34}]bPTH-(3–34) amide and [Nle^{8,18},Tyr^{34}]bPTH-(7–34) amide (Bachem) (20, 26, 28). Antiserum neutralization experiments were carried out with a rabbit polyclonal antiserum (R13) raised against hPTHRP-(1–74) (20); rabbit nonimmune serum and a rabbit polyclonal anti-bPTH-(1–34) antiserum (JH-42, Meloy Laboratories) were used as controls (29). In these experiments, uterus extracts were incubated for 24 hr at 4°C in phosphate-buffered saline with or without the antisera at a final dilution of 1:50 prior to ROS assay (20).

Extracts were examined for PTHRP immunoreactivity in a two-site immunoradiometric assay (IRMA) developed by using affinity-purified, region-specific PTHRP antibodies (30). The assay standard was hPTHRP-(1–74), and nonspecific binding was determined by using an acid/urea extract of adult rat liver (17). Plasma was prepared from blood collected on ice in heparinized tubes containing aprotinin (500 units/ml), leupeptin (5 μg/ml), pepstatin (5 μg/ml), and EDTA (1 mM) and stored at −70°C (30).

***In Situ* Hybridization Histochemistry.** Circumferential and longitudinal sections of uteri from day-21 rats were snap frozen in embedding medium (HistoPrep, Fisher), and 14-μm cryostat sections were prepared as described (20). Sense and antisense 26-base oligonucleotides corresponding to amino acids 62–70 of the rat PTHRP sequence and with a 50% G+C content were 3′ end-labeled with terminal transferase and deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate (20) and included in the hybridization solution at 10^6 cpm/ml. Sections were incubated overnight at 37°C [7°C above the calculated hybrid melting point of 30°C for 4× SSC/65% formamide (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7)]. The final wash was in 0.1× SSC/0.1% 2-mercaptoethanol at 53°C for 1.5 hr. After initial low-resolution images were obtained on x-ray film (Hyperfilm Bmax, Amersham), slides were dipped in Ilford K5-D emulsion at 45°C, dried, and exposed for 2–4 weeks at 4°C (31). Tissue was stained in Gill's hematoxylin no. 3 (Sigma).

RESULTS

PTHRP mRNA Expression in Pregnant Rat Uterus. Total RNA was prepared from the uteri of timed-pregnant rats, and the time course of PTHRP mRNA expression was examined throughout gestation and into parturition (Fig. 1*A*). The average time of gestation in these animals was 21 days, with parturition generally beginning late on day 21 or during the early morning of day 22.

Although PTHRP mRNA could not be detected in RNA prepared from virgin rat uterus, a low and relatively constant level of PTHRP mRNA was found in samples prepared from the uteri of rats from day 6 through day 18 of gestation. On day 19, the expression of PTHRP mRNA began to rise in an asymptotic fashion, reaching a peak at day 21, which continued into but did not increase further during parturition. This same pattern was observed in a number of experiments with a time-course design (see also Fig. 3*A*), and it was clear that the increase in the steady-state level of PTHRP mRNA was not temporally related to the process of parturition *per se*. Indeed, when parturition was prevented (and gestation

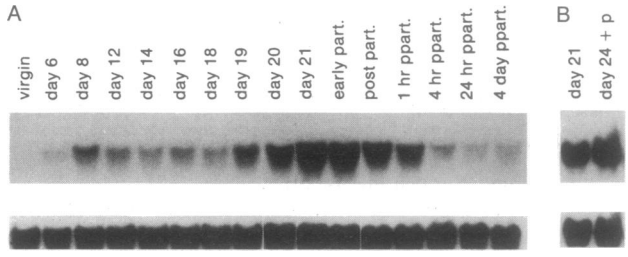

FIG. 1. Expression of PTH mRNA in rat uterus as determined by Northern blot analysis. (*A*) Time course of PTHRP mRNA expression. The early parturition (early part.) sample was obtained from a dam after the delivery of three pups (with eight remaining undelivered), the postpartum (post part.) sample was obtained within 5 min after the delivery of the last pup of the litter, and the other postpartum (ppart.) samples were obtained at the times shown. (*B*) Prolongation of gestation by progesterone. The day-21 sample shown for comparison is representative of the largest PTHRP signal observed in any specimen obtained during normal gestation. The mean pup weight in the progesterone-treated animals at day 24 was 7.4 g as compared to a mean pup weight of 4.3 g at day 21. The filters were probed with a rat PTHRP cDNA fragment (upper gels) (8) and subsequently reprobed with a chicken β-actin cDNA fragment (lower gels) (22). These findings were reproduced in at least three samples prepared from each time point; the apparent modest increase in PTHRP mRNA at day 8 in this figure was not seen in other day-8 animals.

prolonged) by administering progesterone (21), the level of PTHRP mRNA appeared to increase even further (Fig. 1*B*). Progesterone itself had no direct influence on PTHRP mRNA levels when administered beginning on day 17 of gestation (data not shown).

Following parturition, PTHRP mRNA declined rapidly, with a clear decrease being observed by 1 hr postdelivery and a return to the level seen in early pregnancy by 4 hr (Fig. 1*A*). This rapid turnover of PTHRP mRNA has been observed previously in mammary tissue *in vivo* (8) as well as in cultured cells *in vitro* (24).

PTH mRNA was undetectable by RNase protection assay in uterine RNA at any point during gestation (data not shown). A low level of PTHRP mRNA was identified in placental RNA by RNase protection assay, but this level did not change between days 15 and 21 (data not shown).

PTHRP Biological Activity and Immunoreactivity. Tissue extracts were examined for PTHRP bioactivity by using the ROS adenylate cyclase assay (20, 26) and for immunoreactivity by using a two-site IRMA (30). There was an excellent correspondence between the results of these two assays (Table 1) as well as between these measures of PTHRP content and the level of expression of PTHRP mRNA in the uterus on a given day of gestation. In the ROS assay, day-21 uterus extract exhibited a dose–response curve that closely paralleled that of the synthetic hPTHRP-(1–74) standard, and activity was completely eliminated by inclusion of the competitive inhibitor [Nle^{8,18},Tyr^{34}]bPTH-(3–34) amide or [Nle^{8,18},Tyr^{34}]bPTH-(7–34) amide, at 10 μM (20, 28) (data not

Table 1. PTHRP biological activity and immunoreactivity in tissue extracts

Tissue	Time	ROS assay, pmol/g of protein	IRMA, pmol/g of protein
Uterus	Day 15	58	43
Uterus	Day 21	725	490
Uterus	Day 22	998	580
Uterus	Day 1 postpartum	210	83
Placenta	Day 21	20	25

Results are expressed as a function of the protein content in the extracts. The postpartum sample was obtained 8 hr after delivery.

shown). In addition, preincubation of the extract with an anti-PTHRP antiserum (20, 30) resulted in a complete loss of activity, whereas preincubation with nonimmune serum or with an anti-PTH antiserum was without effect (Fig. 2).

PTHRP was undetectable by IRMA (<1.6 pM) in the peripheral circulation of day-21 and day-22 pregnant animals and below or at the limit of detection in a pool of uterine vein samples obtained from day-21 animals. Thus, the peptide did not appear to be secreted from the uterus in significant quantities. PTHRP was also undetectable by IRMA in plasma obtained from the fetus.

Site of PTHRP mRNA Expression. We determined the site of PTHRP expression in the uterus in several ways. Initially, we dissected the uterus from day-19 to day-21 animals into different anatomical segments: (*i*) circumferential "units" containing both a placental attachment site and surrounding uterus, (*ii*) isolated placental attachment sites, and (*iii*) uterine segments lacking placental attachment sites. The PTHRP signal was found to be enriched in the RNA samples prepared from the uterine segments as compared to the placental attachment sites (Fig. 3*A*). By scraping the endometrium from the underlying myometrium, we found that the endometrium was essentially devoid of PTHRP mRNA, whereas the myometrium was highly enriched (Fig. 3*B*).

We also carried out *in situ* hybridization histochemistry on day-21 uterine specimens by using sense and antisense oligonucleotides corresponding to amino acids 62–70 of the PTHRP sequence (20). The antisense probe labeled both the longitudinal (outer) and circular (inner) layers of myometrium, and the silver grains were noted to be predominately perinuclear (Fig. 4*A*). The sense probe gave only background labeling (Fig. 4*B*). Endometrium was not labeled by the antisense probe (Fig. 4*C*).

Control of PTHRP mRNA Expression. In a small percentage of rats, implantation occurs in only one of the two uterine horns, resulting in a unilateral pregnancy. As shown in Fig. 5*A*, the level of PTHRP mRNA in the unoccupied (nongravid) horn of such animals at day 21 was either at "baseline" (i.e., equivalent to that seen in early pregnancy, lane 4) or very much reduced (lane 2) relative to the levels observed in the occupied (gravid) horn (lanes 1 and 3). The possibility existed that the unimplanted uterine horn might be somehow abnormal, so we reproduced these findings experimentally by performing a unilateral tubal ligation in order to achieve implantation in only one horn. As shown in lanes 5 and 6 of Fig. 5*A*, PTHRP mRNA in the unoccupied horn was at

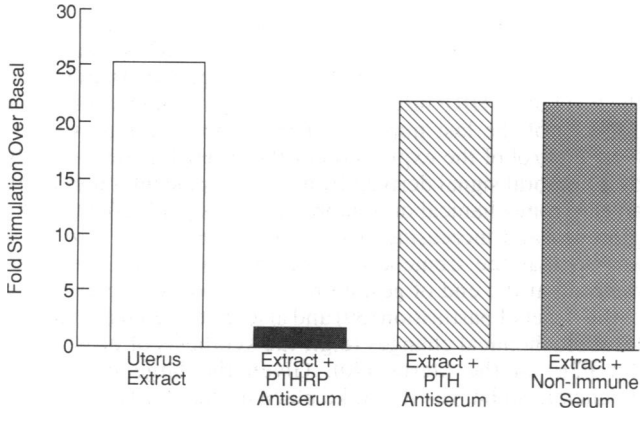

FIG. 2. Immunoneutralization of PTHRP bioactivity by an anti-PTHRP antiserum. The ROS assay results were expressed as the fold stimulation over basal activity (20, 26), which in this assay was 224 cpm. An acid/urea extract of day-21 uterus was assayed alone or in the presence of 1:50 final dilutions of anti-PTHRP or anti-PTH antiserum or nonimmune rabbit serum. The quantity of protein added to each well was 200 μg.

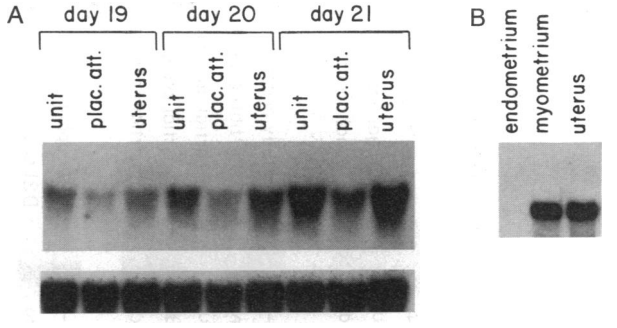

FIG. 3. Localization of PTHRP mRNA expression in rat uterus. (*A*) Comparison in late pregnancy of PTHRP mRNA expression in sampling units containing a placental insertion site and surrounding uterus (unit), in placental attachment sites alone (plac. att.), and in uterine segments dissected free of placental insertion sites (uterus). PTHRP mRNA was determined by Northern blot analysis, as described in Fig. 1. (*B*) PTHRP mRNA expression in endometrium and myometrium as determined by RNase protection analysis. Uterine segments from a day-21 animal were dissected free of placental insertion sites, and the endometrium and myometrium were separated by scraping and were snap frozen. The absence of muscle cells in the endometrial sample was confirmed histologically. RNA was also prepared from a contiguous uterine segment.

baseline (lane 6) as compared to a large day-21 signal in the occupied horn (lane 5).

In several animals, we found bilateral implantation, but one horn was occupied by a single fetus at the cervical end. We obtained tissue specimens separated by ≈1 cm of normal-appearing uterus from the occupied and unoccupied segments of such uterine horns in two animals, one at day 19 and the second at day 21. At day 19, the PTHRP signal in the unoccupied segment was at baseline (Fig. 5*B*, lane 3), whereas the signal in the occupied portion (Fig. 5*B*, lane 2) was equivalent to that seen in a sample from the contralateral, fully occupied horn (Fig. 5*B*, lane 1). At day 21, the PTHRP signals in the occupied (Fig. 5*B*, lane 4) and unoccupied (Fig. 5*B*, lane 5) segments were approximately equivalent.

Taken together, these findings suggest that the factor(s) influencing PTHRP expression in the myometrium is local and dependent upon the presence of the fetoplacental unit but is also capable, late in gestation, of stimulating the expression

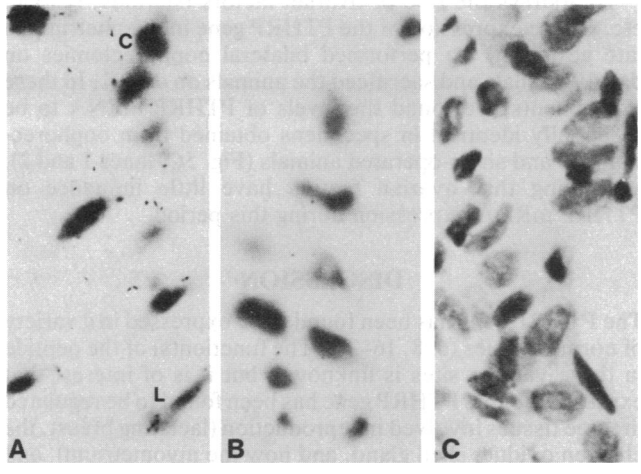

FIG. 4. *In situ* hybridization histochemistry of PTHRP mRNA in day-21 uterus. (*A*) Myometrium was incubated with the antisense probe. (*B*) Myometrium was incubated with the sense probe. (*C*) Endometrium was incubated with the antisense probe. Nuclei of cells of the longitudinal (L) and circular (C) myometrial layers are indicated. The silver grains are located in the cytoplasm in a predominately perinuclear pattern; only the nuclei stain with the hematoxylin. Photomicrographs were taken at ×400 and are printed at ×450.

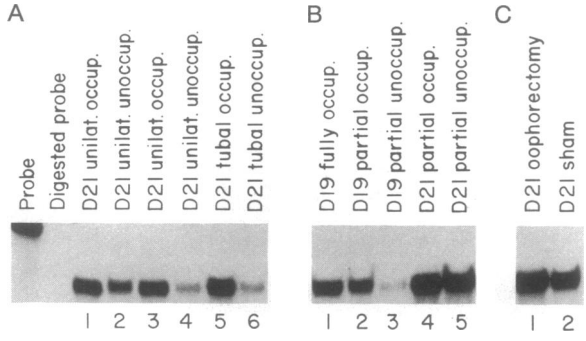

FIG. 5. PTHRP mRNA is expressed in pregnant uterus as a function of intrauterine occupancy. Samples were analyzed by RNase protection assay. The antisense RNA probe (Probe) contains polylinker sequences from the vector and is about 390 bases in length, and the lane containing digested probe in the absence of RNA is indicated. The days of gestation are shown for each sample. (*A*) PTHRP mRNA expression in the uterus of animals with a unilateral pregnancy. RNA was prepared from the occupied (occup.) or unoccupied (unoccup.) uterine horns of rats with spontaneous unilateral (unilat.) pregnancies (lanes 1–4) and from a rat in which a unilateral pregnancy was produced experimentally by ligating the oviduct on one side (tubal) (lanes 5 and 6). (*B*) PTHRP mRNA expression in partially occupied uterine horns. In these animals, occupancy was confined to a single implantation site at the cervical end of a uterine horn on one side, with the contralateral uterine horn being fully occupied. In the day-19 animal, samples were prepared from the fully occupied horn (lane 1) as well as from the occupied (lane 2) and unoccupied (lane 3) portions of the partially occupied horn. In the day-21 animal, the samples from the occupied (lane 4) and unoccupied (lane 5) segments of a partially occupied uterine horn are shown. Identical findings were observed in one additional day-19 and one additional day-21 animal. In addition, the day-19 pattern was observed in samples prepared from two day-20 animals. (*C*) Influence of oophorectomy on PTH mRNA expression in the uterus in late pregnancy. Bilateral oophorectomy or a sham procedure was performed on the afternoon of day 19, and animals were sacrificed on the morning of day 21. The sample shown in lane 1 was obtained from an animal just beginning of deliver the first pup. The slight apparent increase in PTHRP mRNA in the sample from the oophorectomized animal was not seen in three other experiments.

of the mRNA in tissue at some distance from the fetoplacental unit, possibly by diffusion or by the spread of an electromechanical signal.

To address the role of ovarian factors (steroids, relaxin, etc.) in the expression of the PTHRP gene in myometrium in late gestation, we performed bilateral oophorectomies on day-19 animals and sacrificed the animals on day 21. In these experiments, we found the levels of PTHRP mRNA to be essentially identical in specimens obtained from oophorectomized and sham-operated animals (Fig. 5*C*, lanes 1 and 2), suggesting that ovarian factors have little influence on PTHRP mRNA expression during this period.

DISCUSSION

The PTHRP gene has been found to be expressed in a variety of normal tissues (6, 8, 16–20). The function(s) of the peptide in these various sites is unknown, but it is of interest that expression of the PTHRP gene has been found to be regulated in three tissues involved in reproduction (lactating breast, the chicken oviduct shell gland, and now the myometrium), and the production of the PTHRP in two of these tissues (lactating breast and chicken oviduct) has led to the proposal that the peptide may participate in local calcium translocation (8, 18). As might be anticipated by both its widespread expression and the increasing evidence for its regulated expression in different physiological states, the PTHRP gene seems to be subject to a variety of controls. For example, PTHRP mRNA levels have been reported to be influenced by glucocorticoids, vitamin D, prolactin, epidermal growth factor, and phorbol esters (24, 32–34). In addition, PTHRP mRNA bears in its 3′ untranslated region multiple copies of an AUUUA "instability" motif (35) and has been found to have a short half-life (8, 24, 34, 36). This may represent a means of rapidly dissipating a PTHRP biological effect, and it may also be an important posttranscriptional mechanism for regulating the level of expression of PTHRP mRNA (36).

In the present study, we demonstrate that the PTHRP is a product of pregnant rat myometrium. Although present throughout gestation, levels of both PTHRP mRNA and bioactive PTHRP increase sharply during the 48 hr leading up to parturition and fall rapidly following delivery. PTHRP mRNA expression is dependent on intrauterine occupancy and does not appear to be influenced by ovarian factors. In addition, the PTHRP produced in late-term myometrium does not seem to be released into the general circulation. Taken together, these data provide additional support for the proposed autocrine/paracrine nature of the peptide, and they further indicate that PTHRP gene expression is locally regulated in the uterus. Although myometrial cells are not regarded as classical secretory cells, the myometrium has been previously shown to produce other peptides such as prolactin (37).

In spite of the large peak in PTHRP production in the myometrium during the antepartum period, we were unable to detect the peptide in the systemic circulation or in uterine vein plasma by using a sensitive method (30). This suggests that the peptide is rapidly degraded locally or is secreted in a site that is not in free communication with the uterine circulation. This observation is reminiscent of recent data concerning the rapid and transient expression of the PTHRP in lactating breast (8, 32), in that in this setting also the peptide does not appear to reach the systemic circulation (38) but rather is deposited in milk (30, 38). On the basis of these findings, it is quite possible that the only circumstance in which the PTHRP enters the peripheral circulation in sufficient quantity to exert a conventional endocrine effect is when it is produced by tumors associated with the syndrome of humoral hypercalcemia of malignancy. In this circumstance, the PTHRP appears to interact or cross-react with classical PTH receptors in bone and kidney (9).

Our findings indicate that the preterm peak in myometrial PTHRP mRNA expression is a direct function of intrauterine occupancy since this peak was confined to the gravid horn in both naturally occurring and experimental unilateral pregnancy. In further support of this hypothesis, we found that the prolongation of pregnancy by the administration of progesterone permitted further fetal growth *in utero* and resulted in a relatively higher level of PTHRP mRNA than that observed prior to normal parturition (Fig. 1*B*). In addition, the level of PTHRP mRNA decreased very rapidly following delivery of the last pup. All of these observations suggest local control of the expression of the PTHRP gene, possibly by a humoral signal derived from the fetoplacental unit or by an electromechanical or humoral uterine signal reflecting the presence and increasing size of the fetoplacental unit. It is known that the tremendous stretch imposed on the myometrium at the end of gestation is an important determinant of the onset of parturition (39) and also that stretch can induce local biochemical changes (e.g., the synthesis of prostaglandin F2α) in the uterus (40). Given the large number of biochemical changes that take place in the uterus during the antepartum period, it is possible that multiple variables may influence the peak in PTHRP gene expression that occurs at this time.

Mineralization of the fetal skeleton occurs principally during the last trimester of pregnancy at which time a calcium gradient exists between mother and fetus as a result of placental calcium transport (41). Rodda *et al.* (42) have

reported that the PTHRP (but not PTH) is capable of stimulating the placental calcium pump and have proposed that the fetal parathyroids might be the source of the PTHRP that mediates this action in the later stages of gestation. However, the growth rate and mineral demands of the developing fetus are essentially exponential from day 16 onward in the rat (43), so the time course of PTHRP production in the myometrium we report here does not correlate well with the period of peak placental calcium transport. In addition, placental insertion sites were relatively unenriched in PTHRP mRNA as compared to the surrounding myometrium. Nevertheless, in view of the biological findings of Rodda *et al.* (42), a potential involvement of uterine PTHRP in mediating placental calcium transport remains an attractive hypothesis.

Alternatively, the timing of the peak in PTHRP production and its location in the myometrium suggest that the peptide might somehow be involved in the physiological changes associated with labor. The myometrium in all species is relatively quiescent throughout most of pregnancy but during late gestation develops a rhythmic cycle of contraction and relaxation that ultimately expels the products of conception (39, 44). Myometrial contraction is driven by an increase in the intracellular calcium ion concentration, while increases in cyclic AMP contribute to uterine relaxation (45). In PTH-responsive cells, PTHRP elevates cyclic AMP through stimulation of adenylate cyclase (9) and increases cytosolic calcium by means of release from intracellular stores (46). Furthermore, there is evidence that synthetic PTH fragments (47), and more recently synthetic PTHRP fragments (48, 49), are capable of relaxing vascular and nonvascular smooth muscle. Thus, it is possible that the PTHRP may play a role in controlling the rhythmicity and/or force of myometrial contraction in the antepartum period. Since uterine contractions severely depress blood flow (50), another possibility is that the PTHRP might act to dilate uterine blood vessels and assure adequate blood flow to the fetus and uterus during the late stages of pregnancy.

We thank H. Behrman, S. Glasser, R. Hochberg, G. Huszar, K. Insogna, M. Mangin, F. Naftolin, and H. Rasmussen for helpful discussions and advice; C. Morris for technical assistance; B. Wright for providing antiserum JH-42; and N. Canetti for preparing the manuscript. M.A.T. thanks G. Rodan for his support throughout these studies. This work was supported in part by National Institutes of Health Grant AR30102, American Cancer Society Grant PDT-368, and by Merck Sharp & Dohme Research Laboratories.

1. Burtis, W. J., Wu, T., Bunch, C., Wysolmerski, J. J., Insogna, K. L., Weir, E. C., Broadus, A. E. & Stewart, A. F. (1987) *J. Biol. Chem.* **262,** 7151–7156.
2. Moseley, J. M., Kubota, M., Diefenbach-Jagger, H., Wettenhall, R. E. H., Kemp, B. E., Suva, L. J., Rodda, C. P., Ebeling, P. R., Hudson, P. J., Zajac, J. D. & Martin, T. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5048–5052.
3. Stewart, A. F., Wu, T., Goumas, D., Burtis, W. J. & Broadus, A. E. (1987) *Biochem. Biophys. Res. Commun.* **146,** 672–678.
4. Strewler, G. J., Stern, P. H., Jacobs, J. W., Eveloff, J., Klein, R. F., Leung, S. C., Rosenblatt, M. & Nissenson, R. A. (1987) *J. Clin. Invest.* **80,** 1803–1807.
5. Suva, L. J., Winslow, G. A., Wettenhall, R. E. H., Hammonds, R. G., Moseley, J. M., Diefenbach-Jagger, H., Rodda, C. P., Kemp, B. E., Rodriguez, H., Chen, E. Y., Hudson, P. J., Martin, T. J. & Wood, W. I. (1987) *Science* **237,** 893–896.
6. Mangin, M., Webb, A. C., Dreyer, B. E., Posillico, J. T., Ikeda, K., Weir, E. C., Stewart, A. F., Bander, N. H., Milstone, L., Barton, D. E., Francke, U. & Broadus, A. E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 597–601.
7. Thiede, M. A., Strewler, G. J., Nissenson, R. A., Rosenblatt, M. & Rodan, G. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4605–4609.
8. Thiede, M. A. & Rodan, G. A. (1988) *Science* **242,** 278–280.
9. Orloff, J. J., Wu, T. & Stewart, A. F. (1989) *Endocr. Rev.* **10,** 476–495.
10. Mangin, M., Ikeda, K., Dreyer, B. E. & Broadus, A. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2408–2412.
11. Suva, L. J., Mather, K. A., Gillespie, M. T., Webb, G. C., Ng, K. W., Winslow, G. A., Wood, W. I., Martin, T. J. & Hudson, P. J. (1989) *Gene* **77,** 95–105.
12. Yasuda, T., Banville, D., Hendy, G. N. N. & Goltzman, D. (1989) *J. Biol. Chem.* **264,** 7720–7725.
13. Mangin, M., Ikeda, K., Dreyer, B. E. & Broadus, A. E. (1990) *Mol. Endocrinol.* **4,** 851–858.
14. Vasicek, T. J., McDevitt, B. E., Freeman, M. W., Fennick, B. J., Hendy, G. N., Potts, J. T., Jr., Rich, A. & Kronenberg, H. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2127–2131.
15. Habener, J. F., Rosenblatt, M. & Potts, J. T., Jr. (1984) *Physiol. Rev.* **64,** 985–1053.
16. Merendino, J. J., Insogna, K. L., Milstone, L. M., Broadus, A. E. & Stewart, A. F. (1986) *Science* **231,** 388–390.
17. Ikeda, K., Weir, E. C., Mangin, M., Dannies, P. S., Kinder, B., Deftos, L. J., Brown, E. M. & Broadus, A. E. (1988) *Mol. Endocrinol.* **2,** 1230–1236.
18. Thiede, M. A. & Leach, R. (1989) *J. Bone Min. Res.* **4,** S135 (abstr.).
19. Drucker, D. J., Asa, S. L., Henderson, J. & Goltzman, D. (1989) *Mol. Endocrinol.* **3,** 1589–1595.
20. Weir, E. C., Brines, M. L., Ikeda, K., Burtis, W. J., Broadus, A. E. & Robbins, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 108–112.
21. Fuchs, A.-R. (1973) in *Endocrine Factors in Labor*, eds. Klopper, A. & Gardner, J. (Cambridge University Press, Cambridge, U.K.), pp. 163–185.
22. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) *Cell* **20,** 95–105.
23. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
24. Ikeda, K., Lu, C., Weir, E. C., Mangin, M. & Broadus, A. E. (1989) *J. Biol. Chem.* **264,** 15743–15746.
25. Stewart, A. F., Insogna, K. L., Goltzman, D. & Broadus, A. E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1454–1458.
26. Rodan, S. B., Insogna, K. L., Vignery, A. M. C., Stewart, A. F., Broadus, A. E., D'Souza, S. M., Bertolini, D. R., Mundy, G. R. & Rodan, G. A. (1983) *J. Clin. Invest.* **72,** 1511–1515.
27. Stewart, A. F., Elliot, J., Burtis, W. J., Wu, T. & Insogna, K. L. (1989) *Endocrinology* **124,** 642–648.
28. Goldman, M. E., Chorev, M., Reagan, J. E., Nutt, R. F., Levy, J. J. & Rosenblatt, M. (1988) *Endocrinology* **123,** 1468–1475.
29. Wright, B. S., Tyler, G. A., O'Brien, R., Caporale, L. H. & Rosenblatt, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 26–30.
30. Burtis, W. J., Brady, T. G., Orloff, J. J., Ersbak, J. B., Warrell, R. P., Jr., Olson, B. R., Wu, T., Mitnick, M. E., Broadus, A. E. & Stewart, A. F. (1990) *N. Engl. J. Med.* **322,** 1106–1112.
31. Card, J. P., McElligott, S., Gozes, I. & Baldino, F. (1988) *Cell Tissue Res.* **252,** 307–315.
32. Thiede, M. A. (1989) *Mol. Endocrinol.* **3,** 1443–1447.
33. Rodan, S. B., Wesolowski, G., Ianacone, J., Thiede, M. A. & Rodan, G. A. (1989) *J. Endocrinol.* **122,** 219–227.
34. Lu, C., Ikeda, K., Deftos, L. J., Gazdar, A. F., Mangin, M. & Broadus, A. E. (1989) *Mol. Endocrinol.* **3,** 2034–2040.
35. Shaw, G. & Kamen, R. (1986) *Cell* **46,** 659–667.
36. Ikeda, K., Lu, C., Weir, E., Mangin, M. & Broadus, A. E. (1990) *J. Biol. Chem.* **265,** 5398–5402.
37. Walters, C. A., Daly, D. C., Chapitis, J., Kuslis, S. T., Prior, J. C., Kusmik, W. F. & Riddick, D. H. (1983) *Am. J. Obstet. Gynecol.* **147,** 639–644.
38. Budayr, A. A., Halloran, B. P., King, J. C., Diep, D., Nissenson, R. A. & Strewler, G. J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7183–7185.
39. Csapo, A. I. (1981) in *Principles and Practice of Obstetrics and Perinatology*, eds. Iffy, L. & Kamientzky, H. A. (Wiley, New York), pp. 761–799.
40. Parnham, M. J., Sneddon, J. M. & Williams, K. I. (1975) *J. Endocrinol.* **65,** 429–437.
41. Fisher, D. A. (1986) *J. Clin. Invest.* **78,** 601–611.
42. Rodda, C. P., Kubota, M., Heath, J. A., Ebeling, P. R., Moseley, J. M., Care, A. D., Caple, I. W. & Martin, T. J. (1988) *J. Endocrinol.* **117,** 261–271.
43. Feaster, J. P., Hansard, S. L., Outler, J. C. & Davis, G. K. (1956) *J. Nutr.* **58,** 399–406.
44. Fuchs, A.-R. (1969) *Biol. Reprod.* **1,** 344–353.
45. Carsten, M. E. & Miller, J. D. (1987) *Am. J. Obstet. Gynecol.* **157,** 1303–1315.
46. Civitelli, R., Martin, T. J., Fausto, A., Gunsten, S. L., Hruska, K. A. & Avioli, L. V. (1989) *Endocrinology* **125,** 1204–1210.
47. Mok, L. L. S., Nickols, G. A., Thompson, J. C. & Cooper, C. W. (1989) *Endocr. Rev.* **10,** 420–436.
48. Winquist, R. J., Baskin, E. P. & Vlasuk, G. P. (1989) *Biochem. Biophys. Res. Commun.* **149,** 227–343.
49. Mok, L. L. S., Ajine, E., Martin, T. J., Thompson, J. C. & Cooper, C. W. (1989) *J. Bone Min. Res.* **4,** 433–439.
50. Greiss, F. C., Jr., & Rose, J. C. (1989) in *Biology of the Uterus*, eds. Wynn, R. M. & Jollie, W. P. (Plenum, New York), pp. 69–87.