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Osteogenic Protein-1 mRNA in the Uterine Endometrium

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OP-1, a bone morphogenetic protein (BMP) in the TGF-β superfamily, is expressed at high levels in the kidney and in the endometrium of the uterus of non-pregnant mice. During pregnancy the OP-1 mRNA in the endometrium rapidly declined at 4 dpc. Thereafter, OP-1 transcripts were detected in the trophoblastic giant cells of the placenta and the fetal tissues. The uterine OP-1 mRNA downregulation could be mimicked by administration of 17 β-estradiol but not by progesterone to non-pregnant animals. In contrast, OP-1 mRNA expression in kidneys and ovaries was not affected by pregnancy or estrogen treatment. The selective effect of estrogen on OP-1 mRNA in the uterus suggests that OP-1 expression is regulated by tissue specific mechanisms.

Bone morphogenetic proteins (BMPs) are capable of inducing endochondral bone formation at extra-skeletal sites (1-8). Several BMPs and OP-1 (BMP-7) are present at sites of epithelial-mesenchymal interaction and serve as signaling molecules during mammalian development (9-13). For example, BMP-4 induces ventral mesoderm, limb patterning and odontogenesis in mouse embryos (14-16) and OP-1 initiates chondrocyte maturation of chick sternal chondrocytes in vitro (17). In adult tissues OP-1 can be found in selected tissues and remains prominently expressed in the kidney (18). Organ culture and OP-1 gene knock-out studies indicate that OP-1 plays an essential role during kidney development (19-21).

We have now detected high levels of OP-1 in the uterus which decline steeply during pregnancy. Furthermore, estrogen down regulates OP-1 mRNA in the uterus but not in the kidney.

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MATERIALS AND METHODS

Mouse tissue. Postimplantation embryos were obtained from outbred females mated with F1 males. Noon of the day on which the vaginal plug appears is considered to be 0.5 dpc. Tissues were fixed at 40°C in 4 % paraformaldehyde in phosphate-buffered saline and embedded in paraffin. Serial sections were mounted on silanated glass slides, incubated overnight at 40-45°C, and stored at 40°C until used.

mRNA isolation. Eight week old female mice, strain CD-1, were obtained from Charles River Laboratories, Wilmington, MA. Total RNA from various organs was prepared using the acid-guanidine thiocyanate-phenol-chloroform method (18,22). The RNA was dissolved in TES buffer (10 mM Tris-HCl, 1 mM Na2-EDTA, 0.1 % SDS, pH 7.5) containing Proteinase K (Stratagene, La Jolla, CA; approx. 1 mg proteinase/ml TES) and incubated at 37(C for 1 hr. Poly (A)+RNA was selected in a batch procedure on oligo(dT)-cellulose (Stratagene, La Jolla, CA) in 0.5 M NaCl, 10 mM Tris-HCl, 1 mM Na2-EDTA, pH 7.4 (1 × binding buffer). For the selection of poly(A)+ RNA, total RNA obtained from 1 g of tissue was mixed with approximately 0.1 g of oligo(dT)-cellulose (in 11 ml TES containing 0.5 M NaCl). The tubes containing the RNA and oligo(dT)-cellulose were gently shaken for approx. 2 hrs. Thereafter, the oligo(dT)-cellulose was washed twice in 1 × binding buffer and once in 0.5 × binding buffer (0.25 M NaCl, 10 mM Tris-HCl, 1 mM Na2-EDTA, pH 7.4) and poly(A)+ RNA was eluted with water and precipitated with ethanol.

Northern blot Analysis. Poly(A)+ RNA (5 μg per lane) was electrophoresed on 1.2% agarose formaldehyde gels and blotted overnight onto Nytran membranes (Schleicher & Schuell, Keene, NH). 32P-labeled probe was made from a murine OP-1 cDNA fragment (0.68 kb BstXI-BglI frg.) by random hexanucleotide priming. The membranes were hybridized as described (18).

In situ hybridization. For detection of mouse OP-1 by in situ hybridization OP-1 specific gene probes were prepared as described (10,12). Single-stranded sense and antisense RNA probes were labeled with [^{35}S]-UTP (New England Nuclear, Boston, MA) and subjected to alkaline hydrolysis as previously described (10,12). The slides were treated with RNAse A and exposed on NTB-2 emulsion (Kodak) for 1-3 weeks.

RESULTS

High Levels of OP-1 mRNA Are Expressed in the Uterus of Virgin Mice

A detailed analysis of the urogenital tract of 8 week old mice has revealed that OP-1 mRNA is most abun-

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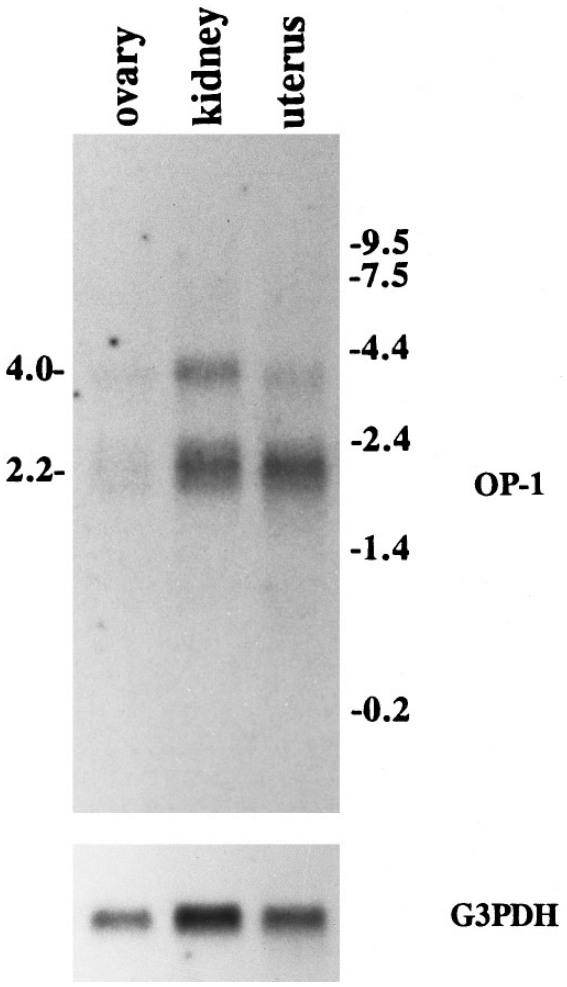

FIG. 1. Relative amounts of OP-1 mRNA in the ovaries, kidneys, and uterus of mice. Equal amounts of poly(A)+ RNA (5μg) from the organs of 8-weeks old mice were run on 1.2% agarose-formaldehyde gels, blotted and analyzed as explained in Methods. Sizes of major OP-1 specific RNA species are shown on the left and an RNA ladder of 0.24-9.49 kb on the right.

dant in the kidney and uterus. OP-1 was also expressed at lower levels in the ovaries (Fig.1). The cellular distribution of OP-1 mRNA in the kidney has been reported (12,19). In the uterus of virgin mice OP-1 transcripts were localized both to superficial epithelium and the subjacent stroma of the endometrium, whereas the myometrium and the surrounding connective tissues did not synthesize OP-1 mRNA (Fig 2).

Differential Expression of OP-1 mRNA in the Kidney, Ovary, and Uterus during Pregnancy

We also analyzed OP-1 mRNA during pregnancy in uterus, kidney and ovary by Northern hybridization of poly(A)+ RNA. The uterine OP-1 mRNA levels became nearly undetectable at 8-dpc and remained low at 13- and 17-dpc (Fig 3) while kidneys and ovaries showed no pregnancy related changes and no changes were observed in the ovaries(data not shown).

17β-Estradiol Is a Negative Regulator of OP-1 Expression

During pregnancy the estrogen and progesterone levels increase many fold and high levels are sustained until birth (23). To determine whether these hormonal changes are responsible for the altered OP-1 transcription in the pregnant uterus, we have subcutaneously administered 17 β-estradiol, or progesterone, or both to nonpregnant female mice.

In the first experiment we attempted to simulate the rapid increase in estrogen and progesterone levels during pregnancy. Nonpregnant mice were injected subcutaneously (sc) on four consecutive days with increasing doses, starting with 20 μg 17β-estradiol, or 100 μg progesterone or the combination of both and doubling the dose on each following day. On the fourth day the animals were sacrificed and mRNA was isolated from uteri and kidneys. A striking inhibitory effect on the uterine OP-1 mRNA expression was observed with 17β-estradiol, whereas progesterone had no effect (Fig 4A). However, OP-1 mRNA levels in the kidneys did not change after 17β-estradiol or progesterone treatment (Fig 4A).

Another experiment addressed the time course: 17β-estradiol was administered at a constant dose of 200 μg (50 μl of 4 mg/ml 17β-estradiol per day, sc. in DMSO [dimethyl sulfoxide] + 150 μl 150 mM NaCl) (Fig 4B). A considerable decrease of OP-1 mRNA 12 hours after its administration and almost undetectable OP-1 expression was found by 48 hr. A modest amount of message reappeared few days later (Fig 4B).

Embryonic and Maternal OP-1 mRNA Transcription

We have also compared OP-1 mRNA levels in the embryo to maternal levels in uterus of 8 week old mice at days 13 and 16 of pregnancy. At a stage of pregnancy when OP-1 mRNA expression in the maternal uterus is almost undetectable the embryonic OP-1 expression is very high, as seen in Figure 5. By in situ hybridization at 8 days of pregnancy OP-1 transcripts were not found in endometrium constituting the maternal placenta as well as in other uterine compartments (data not shown). In contrast, OP-1 mRNA localized to trophoblastic giant cells and fetal tissues as previously reported (12).

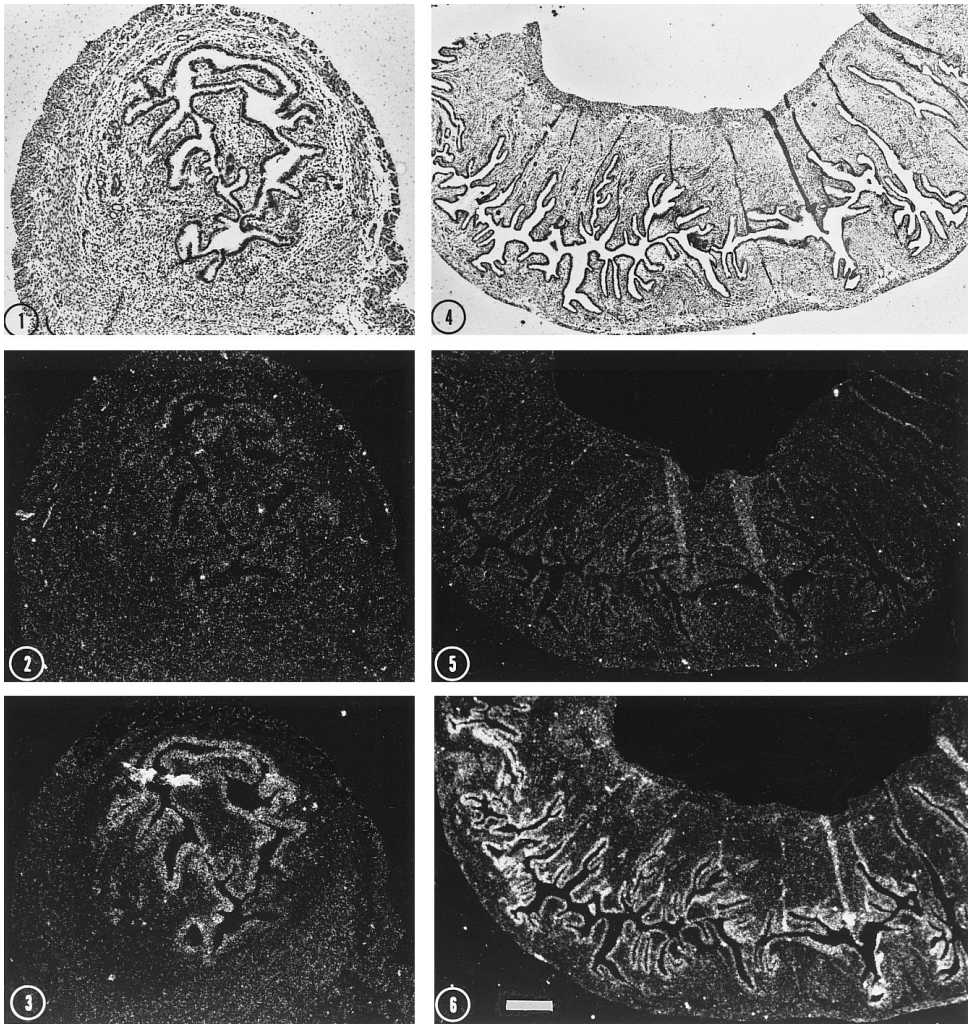

FIG. 2. OP-1 mRNA expression in mouse uterus by in situ hybridization. (Panels 1 and 4) Transverse and longitudinal uterus sections of a virgin mouse visualized by toluidine-blue brightfield image. (Panels 3 and 6) Darkfield image of uterus sections shows transcripts localized in the endometrium along the cavum uteri detected by OP-1 antisense probe. (Panels 2 and 5) Darkfield image using OP-1 sense probe did not yield a signal in adjacent sections. The bar indicates 150 μm in panels 1–3 and 450 μm in panels 4–6.

DISCUSSION

This study identifies the uterus as a site of OP-1 mRNA expression. During pregnancy, however, the OP-1 mRNA in the uterus gradually disappeared. The same effect on the uterine OP-1 transcripts was observed in non-pregnant animals treated with estrogen, suggesting that estrogen is an inhibitor of uterine OP-1 expression. In contrast, no transcriptional OP-1 mRNA changes occurred in the kidney, possibly due to lack of estrogen receptors and/or tissues specific OP-1 promoter elements. We suggest that OP-1 mRNA in the uterus and kidney are regulated independently. Our efforts to find a typical steroid receptor binding site in the genomic DNA sequence (several kilobases) upstream of the OP-1 coding region identified only a related FTZ motif (24) (Ozkaynak et al., unpublished). Hence, the molecular mechanism of estrogen action on OP-1 expression remains unclear. One would expect that endogenous estrogen released during the menstrual cycle would result in fluctuations of the uterine OP-1 mRNA transcription in non-pregnant animals. We have not addressed this in the present study since the animals were not synchronized with respect to estrous cycle and several uteri were pooled for mRNA preparation.

Down regulation of gene expression in uterus by estrogen has also been reported for calbindin-D28k, a vitamin D dependent calcium binding protein (25), and for the expression of the α-subunit of glycopro-

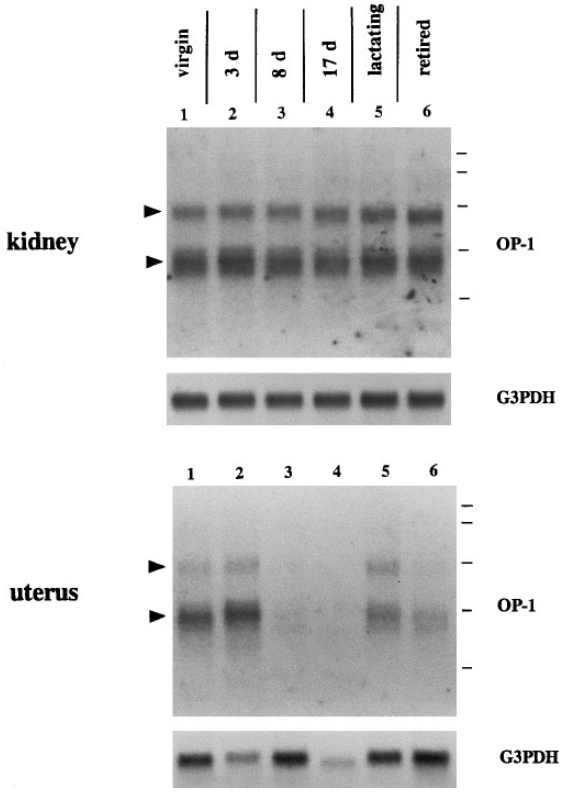

FIG. 3. The effect of pregnancy on OP-1 mRNA expression in kidney and uterus. OP-1 mRNA levels in kidney and uterus before, during and after pregnancy were analyzed by preparing poly(A)+ RNA from these organs and analyzing the OP-1 mRNA by Northern blot hybridization. Kidney poly(A)+ RNA was obtained from individual animals, while the uterus poly(A)+ RNA was pooled from three animals. Lanes 1-6 (kidney top panel and uterus lower panel) represent: lane 1, virgin; lane 2, 3-day pc (post coital); lane 3, 8-day pc; lane 4, 17-day pc; lane 5, lactating for 3-days; lane 6, retired breeders. The arrowheads on the left show the two major OP-1 mRNA species, 2.2 kb and 4.0 kb.

tein hormones (26). Likewise, estrogen treatment in ovariectomized rats leads to a decrease in steady state levels of mRNA for the bone matrix proteins including osteocalcin, α2(I) chain type I collagen, osteonectin, osteopontin, and alkaline phosphatase (27). On the other hand, the acute estrogen depletion in ovariectomized animals results in marked upregulation of IL-6 and IL-11 which could contribute to the postmenopausal osteopenia (28).

While some of these effects seem paradox in view of the well known beneficial action of estrogen in osteoporotic patients estrogen is thought to operate through maintaining the balance between bone formation and resorption (29, 30). These data collectively suggest that estrogen has an important role in balancing the synthesis of structural and secreted proteins both in bone and uterus. Whether estrogen affects the expression of OP-1 in bone in the same way as in the uterus needs to be explored.

Subsequent to the reduction of OP-1 expression in the pregnant uterus high levels of OP-1 message were found in the mouse embryo at 13- and 16-days. Likewise, high levels of OP-1 mRNA were found in giant trophoblasts of the fetal placenta both in vivo and in culture (32). Treatment of cultured trophoblasts with OP-1 revealed that OP-1 inhibits the secretion of the chorionic gonadotropin, but has no influence on the release of estrogen and progesterone by placental trophoblasts (32). Therefore, OP-1 may have a role in regulating the hormonal activity of fetal trophoblasts, which in turn are involved in development of the embryo. Another important role of OP-1 is related to its specific temporal and spatial expression during development in several tissues such as kidney, limb, heart, teeth, intestine, skeletal envelopes and brain (11-13). We conclude that in the uterus estrogen can regulate the OP-1 expression.

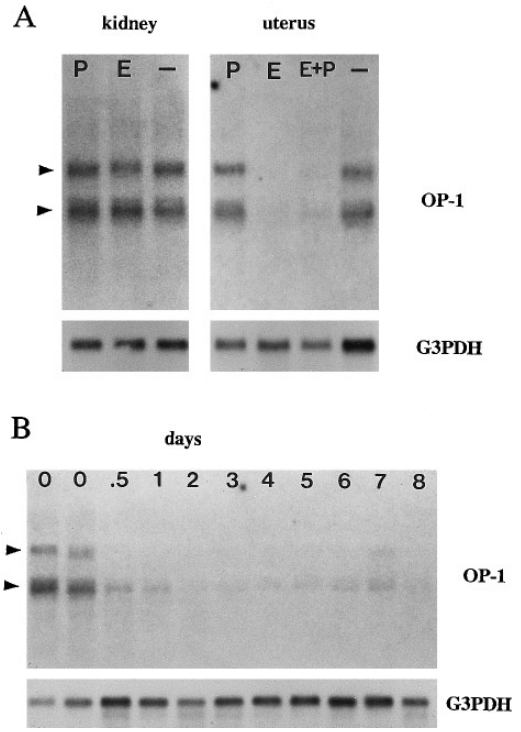

FIG. 4. (A) Effect of 17β-estradiol and progesterone on OP-1 mRNA expression in uterus and kidneys. 17β-estradiol and progesterone were injected sc for four days. The animals were sacrificed on day 5. Left panel kidney, right panel uterus. Kidney: p, progesterone; e, 17β -estradiol; (−) cont., vehicle control (40 μl DMSO and 200 μl 150 mM NaCl per day). Uterus: p, progesterone; e, 17β-estradiol; p + e, progesterone + 17β-estradiol, (−) cont., negative control (40 μl DMSO and 200 μl 150 mM NaCl per day). (B) Time course of estrogen mediated regulation of OP-1 mRNA in the uterus. Animals were injected with 17β-estradiol daily. From left to right, 0-day (negative control), 0.5-, 1-, 2-, 3-, 4-, 5-, 6-, 7-, and 8-days. Arrowheads mark the OP-1 mRNA species.

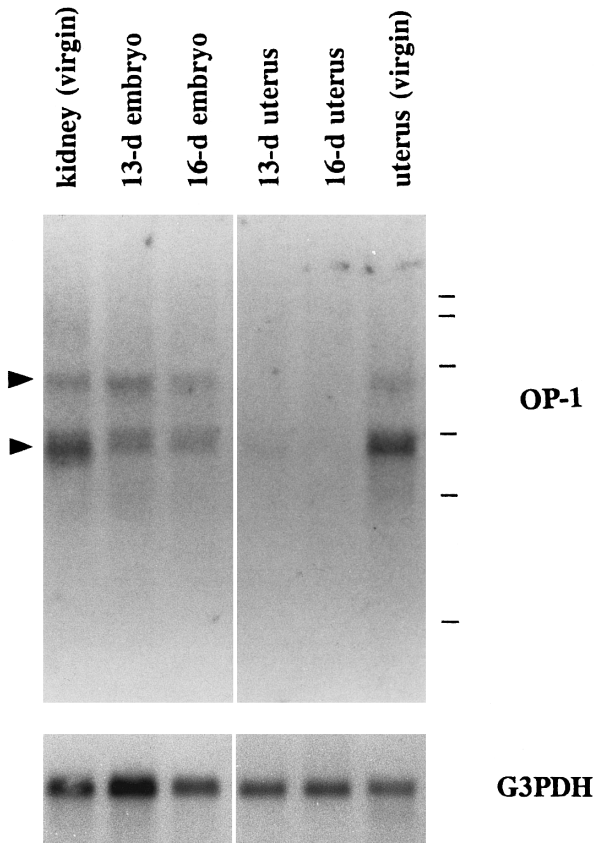

FIG. 5. OP-1 mRNA in the kidney, embryo, pregnant and virgin uterus. From left to right: kidney, 13-d embryo, 16-d embryo, 13-d pc uterus, 16-d pc uterus, and virgin uterus. Arrow- heads mark two major OP-1 mRNA species with 0.24-9.49 kb RNA ladder on the right.

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