Expression of estrogen, progesterone and androgen receptors in the oviduct of developing, cycling and pre-implantation rats

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Abstract

To determine expression and localization of receptors for estrogen (ER), progesterone (PR) and androgen (AR), detailed immunohistochemical evaluations were performed in the Sprague–Dawley rat oviduct during pre- and neonatal development, estrous cycle and pre-implantation period. In addition, real-time RT-PCR studies were conducted to evaluate changes in ERα, ERβ, total PR (PR-A+B), PR-B and AR mRNA expressions. All receptors except for ERβ were detected in epithelial, and stromal or mesenchymal cells of the fetal and neonatal oviduct, and increased with development. During the estrous cycle and early pregnancy, ERα and PR-A+B were expressed in epithelial, stromal and muscle cells throughout the oviduct region, and showed changes in expression predominantly in the isthmus. Only a few epithelial cells in the infundibulum (INF) and ampulla (AMP) showed ERβ staining. AR was detected in stromal and muscle cells throughout the oviduct region, and in epithelial cells of the INF/AMP. Taken together, ERα, PR-A+B and AR were detected in the epithelium of the INF/AMP region, but all of these receptors were expressed in a distinct subset of epithelial cells which were negative for β-tubulin IV, a ciliated epithelial cell marker. These results contribute to a better understanding of the respective roles of ERs, PRs and AR in the rat oviduct.

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Introduction

The mammalian oviduct, or fallopian tube, is an organ known as the female reproductive tract that has a fundamental role in gamete transport, fertilization and subsequent early embryo development. Functions of the oviduct, as well as those of uterus and vagina, are believed to be regulated by two ovarian sex steroid hormones, estrogen and progesterone (P4) (Jansen 1984, Harper 1994). In most tissues, estrogen and P4 actions are mediated by estrogen receptor α (ERα) and estrogen receptor β (ERβ), and P4 receptor-A (PR-A) and P4 receptor-B (PR-B) respectively, belonging to the nuclear receptor superfamily of ligand-inducible transcription factors (Mangelsdorf et al. 1995). Evaluation of the expression and localization of these receptors is key in clarifying the mechanisms of estrogen and P4 actions on cell proliferation, cytodifferentiation and functional differentiation of the reproductive tissues. In the uterus and vagina, ERs and PRs expressions have been well documented. The use of ER or PR gene knockout (KO) mouse tissue has strongly suggested the importance of epithelial–stromal tissue/cell interaction in cell proliferation, cytodifferentiation and

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functional differentiation of uterine and vaginal cells (Cooke et al. 1997, 1998, Buchanan et al. 1998, Kurita et al. 2000a,b, 2001). However, few data regarding the expression and regulation of these receptors in the oviducts of mammals, except for humans and nonhuman primates, have been accumulated (Brenner & Slayden 1994). Neither has there been evaluation of the role of ERs and PRs using ER or PR KO mice. The reason for the lack of information about rodent oviductal ERs and PRs may be the difficulty of examination because of its smaller size and more complex morphological features compared with the uterus, which has no regional differences and only a single epithelial cell type. In contrast, the oviduct has a coiled structure and is composed of four different regions: the infundibulum (INF), ampulla (AMP), isthmus (IST) and uterotubal junction (UTJ). Depending on the oviductal region, there are at least two types of epithelial cells: ciliated epithelial cells and nonciliated or secretory epithelial cells. Therefore, cell- and region-specificity of ERα, ERβ, PR-A and PR-B expressions should be determined for better understanding of the molecular and cellular mechanisms of estrogen and P4 actions in the rat oviduct. Although some previous reports have revealed ERα and ERβ expressions in the rat oviduct (Saunders et al. 1997, Sar & Welsch 1999, Mowa & Iwanaga 2000a,b, Wang et al. 2000), details of cell- and region-dependency still have not been determined or are varied between the reports. Regarding oviductal PR expression, Li (1994) reported neonatal ontogeny in the mouse, but no reports of rat ontogeny and isoform expression, or in cycling and pre-implantation mice and rats were found.

Androgens have uterotrophic effects in intact and ovariectomized immature female rats (Armstrong et al. 1976), and testicular feminized female (Tfm/Tfm) mice showed impaired reproductive performance (Lyon & Glenister 1980), suggesting the importance of androgens in females as well as males. Androgens are known to exert their effects via androgen receptor (AR), which is another member of the nuclear receptor superfamily (Mangelsdorf et al. 1995), and AR mRNA expression has been reported in endometrium, endometrial glands and myometrium of the rat uterus (Hirai et al. 1994). However, the molecular mechanism of androgen action and the role of AR in the female reproductive tract have not yet been demonstrated. Weihua et al. (2002) have recently reported the essential role of AR in the estrogen-induced uterine epithelial cell proliferation of rats, and indicated that stromal AR amplified the ERα signal by induction of insulin-like growth factor-I, which is known to be produced in stromal cells and induces epithelial cell proliferation in a paracrine fashion. Moreover, direct inhibitory effects of the ERα/AR heterodimer on both ERα and AR transactivational properties have been reported (Panet-Raymond et al. 2000). Accordingly, identification of regional and cellular AR localization may allow a better understanding of not only the role of AR, but also the mechanism of estrogen action in the rat oviduct.

In this report, changes in mRNA expression and protein localization of ERα, ERβ, total PRs (PR-A+B), PR-B and AR were determined during the pre- and neonatal development, estrous cycle, and pre-implantation period in the rat oviduct using real-time RT-PCR and immunohistochemistry. In addition, to identify the epithelial cell types expressing steroid hormone receptors, β-tubulin IV was used as a ciliated epithelial cell marker (Renthal et al. 1993) in double immunohistochemical studies.

Materials and methods

Animals

Male and female Sprague–Dawley rats obtained from Charles River Japan, Inc. (Kanagawa, Japan) were used (13 weeks of age). Animals were housed individually in stainless-steel cages with controlled temperature (23 ± 2 °C) and relative humidity (55 ± 10%), and a 13 h light:11 h darkness cycle (0800–2100). Pellet food (CRF-1; Oriental Yeast Co., Ltd, Tokyo, Japan) and municipal tap water were freely available. The day on which sperm was found in the vaginal smear was designated as gestation day (GD) 0 or prenatal day (PD) 0, and the day when neonates were born was designated as neonatal day (ND) 0. The stage of the regular 4-day estrous cycle was specified by the vaginal smear examination using a light microscope every morning. For each total RNA and tissue preparation, ten oviducts were removed from five ether-anesthetized rats in each group (PD 15 and 19 from female fetuses, ND 0, 3, 5, 7, 10, 15 and 20 from female neonates, metestrus, diestrus,
proestrus and estrus from cycling rats, and GD 0, 1, 2, 3 and 4 from pregnant rats). All animals were maintained in accordance with the Institutional Guidelines for Care and Use of Laboratory Animals.

**Total RNA preparation and real-time RT-PCR**

Procedures for total RNA preparation and real-time RT-PCR were described previously (Okada et al. 2002b). Template total RNA (500 ng) treated with DNase I was reverse-transcribed by using SuperScript II RNase H⁻ reverse transcriptase (Invitrogen Corp., Carlsbad, CA, USA) with oligo(dT)₁₂–₁₈ primer for 50 min at 42 °C and then chilled on ice. An aliquot of generated cDNA was amplified with a pair of primers (ERα, forward 5’CTGACAATCGACGCCAAGA3′ and reverse 5’CAGGCTTCAACAGGACAGAC3′; ERβ, forward 5’CTTGCCACTTGGAAACATC3′ and reverse 5’CCAAAGGTGATTTTATGGCC3′; PR-A+B, forward 5’CTTTTTCCTCTTGGAA AATTG3′ and reverse 5’GTATACACGTAAGCAGCCTTCACAGGACCAGAC3′; AR, forward 5’ACCCCATTTGATGTTAGCGG3′ and reverse 5’TGTTGTCGCCACACAGCAG3′; PR-A+B, forward 5’ACCCTCCATGGCAAACCAAGG3′; PR-B, forward 5’CACCTTGCAACCAGAA3′ and reverse 5’AGTCTTACACATTGATTTTATGGCC3′; and AR, forward 5’ACCCCATTTGATGTTAGCGG3′ and reverse 5’TGTTGTCGCCACACAGCAG3′) derived from rat mRNA sequences (GenBank Accession No. Y00102, U57439, L16922, M20133 and U06637 respectively). Primers for PR-A+B, and PR-B were designed to detect a sequence in the 3’-UTR common to the A and B isoforms, and in the 5’-UTR unique to the B isoform respectively. Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was likewise amplified as an internal control (forward 5’TCTACCCAGCGCAAGTTCAAT3′ and reverse 5’ACCCTCCATGGCAAACCAAGG3′; GenBank M17701). Quantitative real-time PCR was carried out in an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA) using SYBR Green PCR Master Mix reagent (Applied Biosystems) as the detector. PCR cycle parameters were 94 °C for 15 s, 60 °C for 30 s and 72 °C for 60 s, followed by a hold temperature for 10 min at 95 °C. The threshold parameter was set as the cycle at which each fluorescent signal was first detected above background, and the number of template copies present at the start of the reaction was determined at exponential increase by comparison with a standard scale prepared from rat genomic DNA (Clontech Laboratories, Inc., Palo Alto, CA, USA). The expression level of each target gene was calculated by standardizing the target gene copy number with the GAPDH copy number in a sample. Purity and specificity of all products were confirmed by omitting the reverse transcriptase, and by single melting temperature, appropriate size and their sequence. Analysis of results is based on duplicate samples from four independent experiments.

**Antibodies**

A mouse monoclonal antibody against ERα (6F11; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) was used at a dilution of 1:50. A rabbit polyclonal anti-rat ERβ antiserum against a synthesized peptide (CSSTEDSKNESQNLQS) corresponding to the C-terminal amino acid residues 467–485 of rat ERβ protein was generated and affinity-purified as described previously (Hiroi et al. 1999, Okada et al. 2002a), and was used at a dilution of 1:100. A pre-diluted mouse monoclonal antibody against PR-A+B (10A9) was obtained from Immunotech (Marseille Cedex, France). The epitope of the PR antibody is located on the C-terminus of PR, which is a common domain between A and B isoforms. A rabbit polyclonal antiserum against AR (N-20; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and a mouse monoclonal antibody against β-tubulin IV (ONSA16; BioGenex, San Ramon, CA, USA) were used at a dilution of 1:200 and 1:250 respectively. Binding specificity of all antibodies has been previously established (Banerjee et al. 1992, Fisher et al. 1997, Hiroi et al. 1999, Okada et al. 2002b, Pelletier et al. 2000, Weihua et al. 2002).

**Tissue preparation and immunohistochemistry**

Oviducts were fixed with 4% paraformaldehyde in 0·1 M phosphate buffer overnight at 4 °C. Sections cut in paraffin at 4 µm were deparaffinized and rehydrated. Antigen retrieval was performed by autoclaving at 121 °C for 15 min in 10 mM citrate buffer (pH 6·0) for ERα, PR-A+B and AR, or at 121 °C for 10 min in 0·8 M urea for ERβ. Sections were then rinsed in distilled water and treated with 0·3% hydrogen peroxide in methanol for 30 min at room temperature (RT). After rinsing in 0·01%
Triton X-100 in PBS (PBT), sections were blocked in normal sheep serum (Dako Corp., Carpinteria, CA, USA) for 30 min at RT, and then incubated overnight at 4 °C with the anti-ERα antibody or the anti-AR antibody, for 48 h at 4 °C with the anti-PR-A+B antibody. Following treatment with each primary antibody, sections were rinsed in PBT and treated with Simple Stain Rat PO (Nichirei, Tokyo, Japan) for 30 min at RT. After a final PBT wash, sections were treated with 0·01% 3,3'-diaminobenzidine tetrahydrochloride (Dojindo Laboratories, Kumamoto, Japan) in 0·05 M Tris–HCl at pH 7·6 including 0·068% imidazole (Sigma, St Louis, MO, USA) and 0·02% hydrogen peroxide for 5 min at RT.

For double immunohistochemistry, sections stained for ERα, PR-A+B or AR as described above were rinsed in PBT and blocked in normal sheep serum, followed by incubation with the anti-β-tubulin IV antibody overnight at 4 °C. Sections were rinsed in PBT and treated with EnVision/AP (Dako) for 30 min at RT. After rinsing in PBT, they were treated with fuchsin (Dako) including levamisole (Dako) for 5 min at RT.

All sections, except those for ERβ staining, were lightly counter-stained with hematoxylin (Dako A/S, Glostrup, Denmark). Normal mouse IgG (Dako A/S) and normal rabbit immunoglobulin fraction (Dako A/S) were used as negative controls in place of primary antibodies for ERα, PR-A+B, β-tubulin IV, and ERβ and AR stainings respectively, showing no specific immunoreactivity.

Immunohistochemical evaluation and statistical analysis

Sections were examined and photographed using a light-microscope (BX60; Olympus Optical Co., Ltd, Tokyo, Japan) attached to a digital camera (DP50; Olympus). Staining intensity was graded as negative, slight, weak, moderate or marked for ERα, ERβ, PR-A+B and AR immunohistochemistries. At least seven specimens from each of five animals were examined for all investigations.

Student’s t-test or Welch’s t-test were performed in cases of equal variance or unequal variance respectively, after ANOVA between oviduct, and uterus or prostate for comparison of gene expressions between tissues (Fig. 1). Duncan’s multiple comparison test was carried out for changes in mRNA expression of ERα, ERβ, PR-A+B, PR-B and AR in the real-time RT-PCR study (Fig. 2). Data are represented as means ± s.d. and considered significantly different at P<0·05.

Results

Expressions of ERα, ERβ, PR-A+B, PR-B and AR mRNAs in the rat oviduct

Quantitative real-time RT-PCR was employed to evaluate changes in expressions of ERs, PRs and AR mRNAs in the rat oviduct. First, to confirm the primers’ specificity, expression levels for ERα, ERβ, PR-A+B, PR-B and AR mRNAs were determined in the diestrous uterus and prostate, as positive control tissues, in addition to the diestrous oviduct of the rats (Fig. 1). Expressions of ERα, PR-A+B and PR-B were much lower in the prostate than in the oviduct, while those of ERβ and AR were inversely highly in the prostate. The percentage for PR-B against PR-A+B was 33·8% in the uterus, being in agreement with previous reports (Ilenchuk & Walters 1987). Also, similar expression patterns for ERα, ERβ, PRs and AR in the rat prostate have been previously reported (Lau et al. 1998).
In fetal rat oviduct, ERα mRNA was expressed at least from PD 15, and increased by PD 19. After birth, oviductal ERα mRNA continued to increase until ND 3, and was maintained at a high constant level through to ND 20 (Fig. 2A). Expressions of ERα mRNA during the regular 4-day estrous cycle and early pregnancy are also presented. ERα level was significantly lower at estrus than at other stages \( (P<0.05) \). In the pre-implantation oviduct, ERα was low on GD 0, which were similar to those at estrus, and increased significantly from GD 1 to 3. In the pregnant oviduct, the peak of ERα increase was noted on GD 2 \( (P<0.01) \), while, ERβ mRNA was detected in a low and constant manner as compared with ERα throughout the development and physiological changes examined (Fig. 2A).

For PRs, two distinct primer pairs were used to determine isoform-specific change in the rat oviduct, one detected A and B isoforms equally and the other was specific for B isoform. Oviductal PR-A+B and PR-B were equally expressed at low levels from PD 15 to ND 3, and both increased gradually from PD 15 to ND 5 (Fig. 2B and C). PR-A+B then increased markedly until ND 20, but PR-B expression continued to increase moderately from ND 7 to ND 20, resulting in a decrease in the percentage for PR-B to one-tenth to a quarter against PR-A+B. During the estrous cycle, PR-A+B mRNA level was significantly lower at estrus than at other stages \( (P<0.05) \). However, PR-B was not significantly changed throughout the estrous cycle. In the pre-implantation oviduct, both PR-A+B and PR-B expressions were low on GD 0, and increased from GD 1 to 3. Moreover, the percentage for PR-B against PR-A+B on GD 0 (21.5%) increased to 46.6% on GD 2 (Fig. 2C).

AR mRNA was also expressed in the rat oviduct, and increased gradually with development of the pre- and neonatal rats. However, no change was exhibited in the cycling and pre-implantation rats (Fig. 2A).

**Immunohistochemical expressions of ERα and PR-A+B in the prenatal and neonatal rat oviducts**

In the uterus, as positive control tissue, ERα and PR-A+B were detected in nuclei of luminal and glandular epithelia, and stroma and muscle layer (Fig. 3) as reported (Ohta et al. 1993, Wang et al. 2000). With these monoclonal antibodies, ontogeny of immunoexpressions for ERα and PR-A+B in the rat oviduct was determined, and summarized in Table 1. Pre- and neonatal oviducts showed simple tubal structure from PD 15 to ND 5, and subsequently differentiated morphologically into the INF, AMP, IST and UTJ after ND 7. In the prenatal oviduct, immunohistochemical staining for ERα exhibited nuclear signals rated as slight and weak in epithelial cells, and negative and slight in mesenchymal cells on GD 15 and 19 respectively. In the neonatal oviduct, epithelial ERα was exhibited as weak and moderate stainings at birth (ND 0) and from ND 3 to 20 respectively (Fig. 4, left panels). However, some ERα-negative cells were present in the epithelium of the INF/AMP region after ND 10. Stromal cells showed ERα stainings at weak, moderate and marked levels at ND 0, from ND 3 to 10, and ND 15 and 20 respectively, and no marked difference in ERα staining was noted among regions in the oviduct. Moderate ERα staining was also found in muscle cells of the IST/UTJ region from ND 7 to 20. PR-A+B immunoexpression was evident in epithelial cells at slight levels on PD 15 and 19; however, it was not present in mesenchymal cells on either prenatal day (Table 1). Slight epithelial PR-A+B expression continued to ND 5 in the undifferentiated oviduct, and to ND 20 in the INF/AMP region (Fig. 4, middle panels). However, some epithelial cells showed negative or moderate PR-A+B signals in the differentiated INF/AMP region from ND 7 to 20. In contrast, epithelial PR-A+B staining was intense in the differentiated IST/UTJ region, and showed moderate and marked signals on ND 7 and 10, and ND 15 and 20 respectively. PR-A+B was also detected in stromal cells after ND 3, but absent in the IST/UTJ region on ND 7 and 10. Muscle cells showed slight and moderate PR-A+B stainings in the IST/UTJ region on ND 10, and ND 15 and 20 respectively (Table 1).

**Immunohistochemical expressions of ERα and PR-A+B in the cycling and pre-implantation oviducts**

ERα and PR immunoreactivities were detected in nuclei of epithelial, stromal and muscle cells of all regions in the diestrous oviduct (Fig. 5). Although most of the stromal cells (INF, AMP, IST and UTJ) and muscle cells (IST and UTJ) were positive for
Figure 2 Changes in expressions of ERα, ERβ and AR (A), and PR-A+B and PR-B (B) in the oviduct of the prenatal, neonatal, cycling and pre-implantation rats. Target mRNA expression levels were evaluated by real-time RT-PCR and standardized to GAPDH mRNA expression. Analysis in duplicate was repeated four times in each experiment. Data are represented as means±S.D. from three independent experiments. **P<0.01 and *P<0.05 vs PD 15, diestrus and GD 0 in developing, cycling and pre-implantation oviduct respectively. Change in the percentage for PR-B against PR-A+B is shown (C).
both ERα and PR-A+B, the number of positive epithelial cells varied depending on the oviduct region. Despite positive stainings for ERα and PR-A+B in all epithelial cells of the IST/UTJ, positive stainings were less in those of the INF/AMP (Fig. 5). Staining intensity of ERα was marked in all cell types of all regions. PR-A+B staining intensity was also marked in stromal and muscle cells of all regions, and in epithelial cells of the IST/UTJ, whereas it was weak in epithelial cells of the INF/AMP. PR-A+B staining in epithelial cells was greater in the IST than in the UTJ (Fig. 5).

Although changes in the staining intensity of ERα and PR-A+B in all regions were weak during the estrous cycle and early pregnancy, epithelial, stromal and muscle cells in the IST/UTJ showed apparent changes, the intensity being higher at diestrus and metestrus than at proestrus and estrus (Fig. 6). In the pre-implantation oviduct, immunolocalization of ERα and PR-A+B exhibited a pattern similar to that in the cycling oviducts. Gestational increases in ERα and PR-A+B staining intensities were observed in the IST/UTJ cells (Fig. 6).

**Immunohistochemical expression of oviductal ERβ**

For immunohistochemistry of ERβ, Weihua et al. (2002) recently reported an improved method to detect the ERβ signal in the rat uterus by using urea in place of citrate buffer as the antigen retrieval buffer, and detected apparent nuclear ERβ stainings in rat uterine epithelial and stromal cells. Effectiveness of the antigen retrieval with urea has also been reported for stainings of ERα, PR, AR and other intranuclear antigens in paraffin sections (Taylor et al. 1994). Therefore, we employed this method for ERβ immunohistochemistry, and could detect ERβ immunoreactivity in nuclei of uterine luminal and glandular epithelial, and peripheral stromal cells in addition to granulosa cells of the ovary, which served as controls (Fig. 7). To confirm our findings of ERβ protein expression in the rat tissues, we employed two commercially available rabbit polyclonal antisera to rat ERβ as reported (Okada et al. 2002a): PA1–310 (Affinity Bioreagents, Inc., Golden, CO, USA) and 06–629 (Upstate Biotechnology, Lake Placid, NY, USA) against amino acid residues 467–485 and 54–71 of rat ERβ respectively. With both ERβ antisera, similar results to those with our antibody were obtained in the ovary and uterus by immunohistochemistry using either citrate buffer or urea (data not shown).

In the diestrous oviduct, however, few ERβ-positive cells were found in epithelial cells of the INF/AMP with our (Fig. 7) and two other ERβ antibodies (data not shown). No ERβ was detected in stromal cells of the INF/AMP, or in any cells of the IST/UTJ of the diestrous oviduct. ERβ immunoreactivity was indistinguishable in the pre- and neonatal oviduct (Table 1), and showed no remarkable change during the estrous cycle and pre-implantation period in the adult oviduct (data not shown).

**Immunohistochemical expression of oviductal AR**

In the adult rat prostate, positive AR was detected in nuclei of glandular epithelial cells (Fig. 3) as previously reported (Husmann et al. 1990).
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PD: prenatal days, ND: neonatal days, Epi: epithelial cells, Str: stromal cells, Mus: muscle cells.

Some epithelial cells showed negative stainings. Some epithelial cells showed negative or moderate stainings.
However, in the developing oviduct, no AR immunoreactivity was detected in Müllerian duct cells on PD 15 and 19. Both epithelial and stromal ARs were first detected at a low level on ND 3, and maintained at a similar level until ND 20 in all differentiated regions (Table 1, and Fig. 4, right panels). In the IST and UTJ, muscle AR was observed at slight and weak levels on ND 7 and 10, and ND 15 and 20 respectively.

In the diestrous oviduct, some AR stainings were detected at weak levels in epithelial cells of the INF/AMP (Fig. 5, right panels). In contrast to ER and PR expressions in all epithelial cells, most epithelial cells did not show AR immunoreactivity in the IST/UTJ. Stainings for stromal and muscle ARs were weak or moderate throughout the oviduct region. However, no remarkable change was observed in the expression and localization of AR during the estrous cycle and early pregnancy.

### Immuno-co-localization of epithelial ERα, PR-A+B and AR with β-tubulin IV

To determine surface epithelial cell types expressing ERα, PRs and AR, double immunohistochemical staining for each receptor with β-tubulin IV, a ciliated epithelial cell marker (Renthal et al. 1993) was performed. In the INF/AMP, the mucosa formed numerous elaborately branched folds and their lumina were labyrinthine systems of narrow spaces between the branching folds covered with the epithelium. The epithelia were basically simple columnar and consisted of two kinds of cells, ciliated and nonciliated epithelial cells. They sometimes appeared pseudostratified in the oblique sections. The ciliated cells were positive for β-tubulin IV and contained round pale nuclei in the apical or middle region of the cells, while the nonciliated cells were negative for β-tubulin IV and had dark nuclei oval to slender in the basal region. Each expression of ERα, PR-A+B and AR was restrictedly observed in nonciliated epithelial cells, but not in ciliated epithelial cells of the INF/AMP (Fig. 8). In contrast, almost all epithelial cells were negative for β-tubulin IV in the UTJ/IST, but positive for ERα and PR-A+B.

### Discussion

Despite extensive research on expression of sex steroid hormone receptors in female reproductive tissues of humans and laboratory rodents, expression, localization and function of these receptors in the oviduct remain unclear. This study demonstrates that ERα, ERβ, PR-A, PR-B and AR are expressed in the rat oviduct throughout physiological conditions in a cell- and region-dependent manner. A lack of these receptors in ciliated epithelial cells of the rat oviductal epithelium was noted.

In the real-time RT-PCR study, expressions of ERα, PR-A+B and PR-B mRNA were detected to be regulated in the fetal, neonatal, cycling and pregnant rat oviduct, suggesting that these receptors may play roles in the physiological functions of the rat oviduct. Oviductal ERβ expression was reported to be low in rats (Saunders et al. 1997, Mowa & Iwanaga 2000a,b) and mice (Couse et al. 1997), while Sar & Welsch (1999) and Jefferson et al. (2000) failed to detect ERβ immunopositive cells in oviduct of rats and mice respectively. In the present study, although ERβ mRNA was detected in the rat oviduct by RT-PCR, it was present only at low constant levels throughout physiological conditions. Immunoreactivity for ERβ was limited to a few nonciliated epithelial cells in the INF/AMP. Thus, abundant ERα may be a major ER subtype and play an essential role in development and function of the rat oviduct. On the other hand, two PR isoforms, PR-A and PR-B, are produced from a single gene by transcription at two distinct promoters (Kastner et al. 1990), and the ratio of isoforms varies depending on the reproductive tissues during development (Shyamala et al. 1990) and estrous cycle (Mangal et al. 1997). In the rat oviduct, the present study demonstrated a definite variation in the mRNA expression of PR isoforms evaluated by the ratio of PR-B to total PR during development and different hormonal circumstances as adults. At present, although little evidence has been available to define the physiological significance of P4 action via PR-A and/or PR-B, it seems highly probable that differential expression of two isoforms in the oviduct is fundamental for the cell growth, differentiation and functions in response to P4. PR-A is reported to be able to act as a transcriptional inhibitor of PR-B when both proteins are co-expressed (Vegeto et al. 1993).

In the developing oviduct, ERα, PR-A+B and AR were expressed in both epithelial and mesenchymal or stromal cells, and the staining
intensity and mRNA level increased with the growth of neonates. During this early postnatal period when increases in these receptors were observed, immunoreactivity for β-tubulin IV in the rat oviduct appeared between ND 5 and ND 7 (data not shown). Komatsu & Fujita (1978) have reported in their electron-microscopic study that differentiation of ciliated cells, which is believed to be elicited by the initiation of endogenous estrogen (17β-estradiol; E2) production, occurred on ND 5 in the mouse oviduct. Furthermore, neonatal estrogen administration accelerated cilia formation.
in the mouse oviduct (Eroschenko 1982). The teratogenic and carcinogenic effects of perinatal exposure to diethylstilbestrol (DES), a synthetic estrogen, on the mouse and rat oviduct are well documented (Herbst & Bern 1981, Newbold et al. 1983, Iguchi 1992), and ERα KO mice showed resistance to those adverse effects of DES (Couse et al. 2001). Therefore, the increase in oviductal

**Figure 5** Immunohistochemical localization of ERα, PR-A+B and AR in the diestrous rat oviduct. In the INF and AMP, ERα (left), PR-A+B (middle) and AR (right) were detected in nuclei of epithelial and stromal cells. However, fewer epithelial cells showed immunoreactivity for ERα, PR-A+B and AR. In the IST and UTJ, ERα, PR-A+B and AR were detected in nuclei of epithelial, stromal and muscle cells, except for epithelial AR. Arrows represent high magnification of AR-positive cells, as shown in the inset in AR-AMP. All immunoreactivity was abolished by incubation with normal mouse or rabbit IgG as controls (data not shown). Bar: 100 µm.
ERα may have a critical role in the neonatal proliferation and cytodifferentiation of oviductal cells in response to endogenous and exogenous estrogens. If estrogen regulates epithelial ciliary formation directly via epithelial ERα in the neonatal oviduct, this potential mechanism may be different from that in ERα-negative epithelial cells of the adult oviduct discussed below. In contrast, although the physiological role of PRs and AR has not been clearly demonstrated in the development of the rat oviduct, both epithelial and stromal cells could be targets for endogenous progestins and androgens, and exogenous PRs and AR modulators. Especially, because epithelial cells of neonatal IST markedly expressed PRs, they could be a critical target for progestin action.

Oviductal cilia are believed to have the critical role in ovum transport through oviduct to uterus in cycling and pregnant rats (Halbert et al. 1989). Estrogen accelerated ovum transport in pregnant rats, and concomitant P4 treatment blocked the estrogen-induced events (Banik & Pincus 1964, Fuentealba et al. 1988). In the cycling and pregnant rats, ovariectomy or hypophysectomy,
and treatment with the aromatase inhibitor 4-hydroxyandrostenedione, caused delayed ovum transport due to reduction of estrogen production (Wu et al. 1971, Forcelledo & Croxatto 1986, 1988). In contrast, treatment with the PR antagonist RU486 caused accelerated ovum transport in rats (Fuentealba et al. 1987). Thus, estrogen and P4 may have roles in ovum transport by regulating oviductal ciliogenesis in rats. In the present study, however, a lack of ERα and PRs in ciliated epithelial cells was shown by double immunostaining with normal mouse and rabbit IgG (Negative). Bar: 20 µm.

**Figure 8** Double immunohistochemical localization of ERα, PR-A+B or AR, with β-tubulin IV in the AMP of the diestrous oviduct. Staining of ERα, PR-A+B or AR (brown), and β-tubulin IV (red) were not found to be co-localized in the same surface epithelial cells. Arrowheads indicate ERα-, PR-A+B- or AR-positive, but β-tubulin IV-negative, cells. Arrows indicate ERα-, PR-A+B- or AR-negative, but β-tubulin IV-positive, cells. Immunoreactivity was abolished by incubation with normal mouse and rabbit IgG (Negative). Bar: 20 µm.

Although effects of androgens on the oviduct still remain obscure, AR was predominantly detected in nonciliated epithelial cells in the INF/AMP, and stromal and muscle cells in the rat oviduct. Recently, a direct inhibitory effect of ERα/AR heterodimer on both ERα and AR transactivation properties has been reported (Panet-Raymond et al. 2000). Although, in the rat oviduct, co-localization of AR and ERα was not determined, both AR and ERα were expressed in stromal, muscle and nonciliated epithelial cells. Interaction of AR and ERα, therefore, may possibly occur in the rat oviduct as well as in the uterus, if AR and ERα co-localize in the identical cell.

In conclusion, the cell- and region-dependent ERα, ERβ, PRs and AR expressions were immunohistochemically determined in the rat oviduct throughout various physiological conditions. This study could be helpful for understanding the molecular and cellular mechanisms underlying estrogen, progestin and androgen actions on the rat oviduct.

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