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Expression of Estrogen Receptor β Is Developmentally Regulated in Reproductive Tissues of Male and Female Mice

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ABSTRACT

By the use of ribonuclease protection assay (RPA) combined with immunohistochemical techniques, the expression of estrogen receptor (ER) α and ERβ was mapped in the developing gonads and reproductive tracts of male and female mice from fetal day 14 to postnatal day 26 (PND 26). This study was designed to determine the pattern of expression of both ER subtypes in specific tissue compartments during development. In ovaries, ERα mRNA was detected at all ages examined; ERβ mRNA was seen as early as PND 1, and its expression increased with age. Immunolocalization showed ERβ in differentiating granulosa cells of the ovary, whereas ERα was predominantly seen in interstitial cells. The remainder of the female reproductive tract showed ERα mRNA at all ages examined with little or no significant levels of ERβ, except on PND 1 when a low level of message appeared. In males, ERα and ERβ mRNA were detected in the fetal testis; however, ERβ gradually increased until PND 5 and subsequently diminished to undetectable levels by PND 26. Immunolocalization showed ERα in the interstitial compartment of the testis, whereas ERβ was seen predominantly in developing spermatogonia. The remainder of the male reproductive tract showed varying amounts of both receptors by RPA and immunostaining throughout development. These studies provide information useful in studying the role of both ER subtypes in normal differentiation, and they provide indications of differential tissue expression during development.

INTRODUCTION

Estrogens are known to regulate cell functions such as growth and differentiation in target tissues via receptor-mediated pathways. Until recently, only a single type of estrogen receptor (ER) was thought to exist and to mediate the genomic effects of estrogens [1]. However, reports have now described a second ER, termed ERβ [2,3], that has been shown to exhibit a tissue distribution distinct from the classical ER (now termed ERα) [4–6]. The biological significance of two ER subtypes is unclear but may provide an explanation for the selective actions of estrogens in different target tissues. The fact that both ERα and ERβ are found in numerous mammalian species suggests that selective effects of estrogens may reside in promoters that are differentially responsive to ERα and ERβ.

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The ontogeny of ERα in the developing reproductive tract of a number of species has been documented for many years [7–10]. The presence of ERβ has only recently been described in adult reproductive tract tissues of mice [4,11], rats [12–14], cows [15], and humans [3,16]. Developmental expression of ERβ has been reported in the prostate [17] and testis [18,19] of the rat, and in the fetal reproductive tract of humans [5]. ERβ has also been reported in several reproductive tract tissues of the mouse during embryogenesis [20]. Our particular interest has been in the expression of the two ER forms in the developing reproductive tract tissues of male and female mice. Since the differentiating mammalian reproductive tract has been shown to be uniquely sensitive to various estrogenic compounds, and exposure either prenatally or neonatally results in long-term abnormalities including neoplasia [21–23], the role of both ERα and ERβ in the induction of these lesions warrants further investigation. Synthetic and naturally occurring xenoestrogens have different relative in vitro affinities for ERβ and ERα receptors, and some ligands preferentially bind to ERα, whereas others bind more efficiently to ERβ [24–26]. Therefore, the presence and relative abundance of both ERα and ERβ in a specific tissue may be a determining factor in the relative effect of a particular compound on that tissue. The present study describes ERα and ERβ mRNA and protein expression in specific tissue compartments of reproductive tract tissues in male and female mice at various stages of normal development and differentiation.

MATERIALS AND METHODS

Animals

Adult CD-1 (Crl:CD-1 [ICR] BR) mice were obtained from Charles River Breeding Laboratories (Raleigh, NC) and bred to male mice of the same strain in the breeding facility at the National Institute of Environmental Health Sciences (NIEHS; Research Triangle Park, NC). Vaginal plug detection was considered day 0 of pregnancy. Pregnant mice were housed under controlled lighting (12L:12D) and temperature (21–22°C) conditions. Mice were provided with NIH 31 laboratory mouse chow (National Institutes of Health, Bethesda, MD, purchased from Zeigler Brothers, Inc., Gardners, PA) and fresh water ad libitum. All animal procedures complied with an approved NIEHS/NIH animal care protocol.

Pregnant mice were killed and fetuses were removed at 14 and 16 days postconception (dpc; plug day = 0 dpc). For the remaining time points, pregnant mice delivered their young on 19 dpc and were randomly standardized to

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8 pups per litter. Mice were killed on days 1, 5, 12, 19, and 26 of life (day of birth = postnatal day [PND] 1).

Reproductive tract tissues were quickly removed and processed by one of two methods: 1) tissues collected for ribonuclease protection assay (RPA) were snap-frozen in liquid nitrogen and stored at −70°C until further processed; 2) tissues collected for immunohistochemical analysis were fixed in formalin for 6 h at 4°C, transferred to 70% ethyl alcohol at 4°C, and processed for histological evaluation. Tissues were embedded in paraffin and serially cut into 4-μm sections.

RNA Isolation and RPA

RNA isolation. For fetal tissue collection, 6 pregnant females were killed at each time point (14 dpc and 16 dpc); the abdominal cavity was opened, and fetuses were quickly removed. Using a dissecting scope, the reproductive tract was collected en bloc into cold PBS, and the gonad was dissected away from the remainder of the reproductive tract. Reproductive tracts and gonads were frozen and pooled from approximately 30 female and 30 male mice for each age. At PNDs 1, 5, 12, 19, and 26, reproductive tracts and gonads were collected into cold PBS, dissected, and frozen. Approximately 8 mice per sex per time point were pooled on PNDs 1, 5, and 12, and 4 mice per sex per time point were pooled on PNDs 19 and 26. Tissues collected from the females included ovary/oviduct and uterus; tissues from the males were testis, epididymis, vas deferens, seminal vesicle, and coagulating gland complex, and the remainder of the reproductive tract including the prostate. For PND 1 males, seminal vesicles were not separated from reproductive tract tissues.

Tissues were pulverized and homogenized with a handheld homogenizer in Trizol (Gibco-BRL, Grand Island, NY), and total RNA was isolated according to the manufacturer's instructions. A small aliquot of the final preparation was used to determine the amount of total RNA by A_{260}, and the integrity of the RNA was checked on 1% agarose gels by viewing ribosomal RNA bands. RNA was frozen at −70°C until further use.

RPA. The probes used in this study have been previously described in detail [4,27]. Antisense riboprobes were generated from linearized templates using Maxiscript reagents (Ambion, Austin, TX), the appropriate RNA polymerase (T3 or T7), and the incorporation of [^{32}P]CTP (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's protocol. The full-length mouse ERα antisense riboprobe was 490 nucleotides (nt) and produced a specific protected fragment of 366 nt [27]. The mouse ERβ antisense riboprobe was generated from the cloned cDNA fragment and was 318 nt in full length and generated a protected fragment of 262 nt [4]. An antisense riboprobe specific for mouse cyclophilin, used to equate loading among lanes, was generated from the template pTRI-cyclophilin (Ambion) at a full length of 165 nt and produced a protected fragment of 103 nt.

For all postnatal endpoints, 10 μg of total RNA was used, and for the fetal end points 2.5 μg was used. All RPA reactions consisted of 5×10^4 cpm of each probe, sample RNA, and yeast tRNA (for a final total RNA equal to 25 μg), which were mixed and ethanol-precipitated at −70°C for a minimum of 3 h to a maximum of overnight. The resulting pellets were then processed through the RPA using the Hybspeed RPA reagents (Ambion) according to the manufacturer's protocol. Final analysis of protected fragments was carried out by electrophoresis on a 1.5-mm-thick, 6% *bis*-acrylamide/8.3 M urea/single-strength Tris-borate-EDTA (TBE) gel (National Diagnostics, Atlanta, GA), which was then fixed, dried, and exposed to a Phosphorimager screen (Molecular Dynamics, Sunnyvale, CA) and then to x-ray film.

ER Immunohistochemistry

ERα. Tissue sections from a minimum of three mice were immunostained for each age and sex. Tissue sections were deparaffinized in xylene, hydrated in a series of graded ethanols, and washed in single-strength automation buffer (AB; Fisher Scientific, Norcross, GA). Sections were then treated with 3% hydrogen peroxide to eliminate endogenous peroxidase. After washes in AB, sections were incubated with 0.015 mg/ml trypsin in AB for 4 min and with 0.025 mg/ml deoxyribonuclease I in AB for 3 min, and washed with AB. Sections were then incubated with normal goat serum (Vector Laboratories, Burlingame, CA) to block nonspecific binding for 20 min. The sections were then incubated with primary antibody, rat anti-human ERα (H222; Abbott Laboratories, Chicago, IL) at a dilution of 1:3 overnight at room temperature. Negative controls were run on adjacent tissue sections with preimmune serum (Abbott Laboratories) or without the primary antibody (buffer only). Sections were then washed with AB and incubated with biotinylated goat anti-rat IgG (Vector Laboratories) for 1 h. After this incubation, sections were washed and incubated with ExtrAvidin peroxidase (Sigma Chemical Company, St. Louis, MO) at a dilution of 1:50 for 30 min. Visualization of the peroxidase was carried out by covering the sections with diaminobenzidine (DAB; Sigma Chemical Company) at 0.5 mg/ml in AB containing 0.01% H_2O_2 for 10 min. Sections were rinsed in distilled water and dehydrated in a graded series of ethanols and xylenes, and coverslipped for evaluation by light microscopy.

ERβ. Adjacent tissue sections of the samples immunostained for ERα were also immunostained for ERβ. Sections were deparaffinized and rehydrated, and endogenous peroxidase was eliminated as described for ERα above. After washes in AB, tissue sections were rinsed with water and microwaved for 5 min in dH_2O for antigen retrieval. Sections were then incubated with 10% BSA in AB for 20 min, then incubated 2 h at room temperature using rabbit anti-mouse ERβ at a dilution of 1:50 in AB pH 6.8 (310; Affinity Bioreagents, Golden, CO). Negative controls of adjacent tissue sections were incubated with anti-mouse ERβ preabsorbed with ERβ peptide (Affinity Bioreagents), or preimmune serum at the same dilution. Sections were then washed with AB and incubated with biotinylated goat anti-rabbit IgG (Vector Laboratories) at a concentration of 1:500 in AB for 1 h. Sections were washed with AB and incubated with ExtrAvidin peroxidase for 30 min, and treated with DAB in AB containing 1% H_2O_2 for 10–20 min for color development. Sections were rinsed in distilled water, dehydrated, coverslipped, and evaluated using light microscopy.

RESULTS

RPA

Two distinct bands differing in size by approximately 15 nt appeared in several tissues although a single protected fragment representative of ERβ mRNA was expected. It is important to note that these bands appeared only in those

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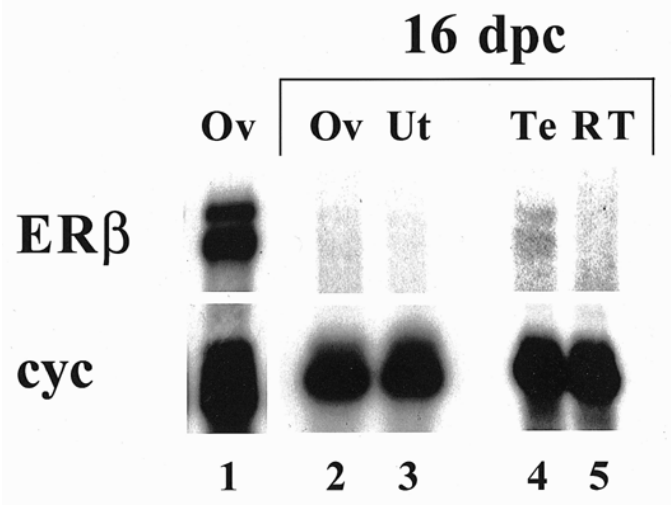

FIG. 1. RPA results of 16 dpc male and female reproductive tract tissues. 2.5 μg of total RNA was used in each RPA for each fetal tissue. Lane 1: PND 19 ovaries (Ov; 10 μg total RNA, positive control); lanes 2–5: ovaries (Ov); uteri (Ut); testes (Te); reproductive tracts (RT). ERβ mRNA was seen in the 16 dpc testis sample only. Cyclophilin (cyc) in each gel demonstrates that the total RNA was comparable for the fetal samples in each gel. The film in this figure was exposed overnight for ERβ in the fetal samples and 8 h for cyclophilin and ERβ in the PND 19 control ovaries.

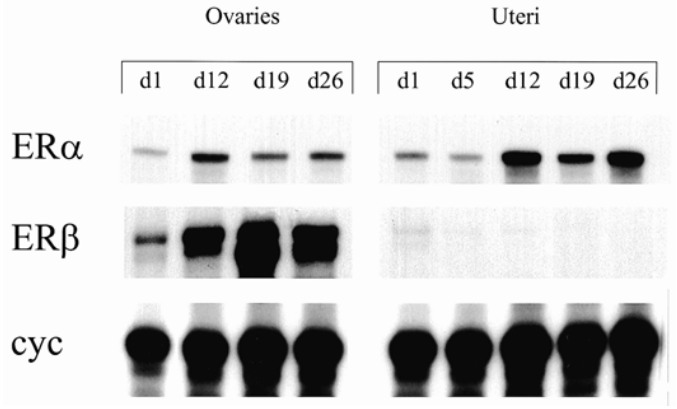

FIG. 2. RPA results for all postnatal female reproductive tract tissues. **Left (Ovaries)** Pooled ovarian RNA (10 μg) from animals on PND 1, 12, 19, and 26 were assayed. The presence of both ERα and ERβ transcripts was apparent in all ovary samples examined, showing an increase in ERβ mRNA with increasing age of the animal. **Right (Uteri)** Pooled uterine mRNA (10 μg) from animals on PND 1, 5, 12, 19, and 26 shows an abundant amount of ERα mRNA and little or no expression of ERβ mRNA with the exception of the PND 1 uteri. Cyclophilin (cyc) in ovarian and uterine samples demonstrates that the total RNA was comparable for all samples compared. The film in this figure was exposed overnight for ERα and ERβ and 8 h for cyclophilin.

tissues that also exhibited positive ERβ immunoreactivity, and therefore both are probably representative of detectable ERβ mRNA. As described previously [2], the ERβ riboprobe was transcribed from a partial cDNA clone of the mouse ERβ gene generated by PCR using primers specific to the rat ERβ gene. Because the rat primers become incorporated into the clone during this process and are not 100% homologous to the corresponding mouse sequences, there exist some degenerate sequences in the termini of the clone and resulting riboprobe. This small difference in the riboprobe and protected mRNA sequence can be partially exploited during the ribonuclease (RNase) step of the assay, resulting in incomplete RNase digestion and the appearance of two bands.

The results of the RPA on the fetal tissues are shown in Figure 1; 16 dpc ovaries and uteri showed no detectable ERβ mRNA, whereas 16 dpc testes did show the presence of ERβ mRNA; the remainder of the male reproductive tract showed no evidence of ERβ transcripts. ERα transcripts were detected in all male and female 16 dpc tissues examined by RPA (data not shown). Total RNA from the 14 dpc testis was positive for ERβ mRNA expression (data not shown).

The results of the RPA on postnatal female tissues are shown in Figure 2. All time points showed the presence of both ERα and ERβ mRNA in the ovaries, with a peak in ERβ mRNA transcripts at PND 19. The uteri showed ERα at all ages examined; a low level of ERβ was seen in the uterus on PND 1 but was not apparent in older tissues. Longer exposure time (2 days) did not reveal ERβ mRNA in the uterus at later ages.

Results of RPA analysis of ERα and ERβ mRNA in male tissues are seen in Figure 3. The neonatal testis showed the presence of ERα that decreased with age starting on PND 19. An abundant amount of ERβ mRNA was seen on PNDs 1 and 5. Although ERβ mRNA was present at later time points, levels were relatively much lower. RPA analysis of the remainder of the reproductive tract showed

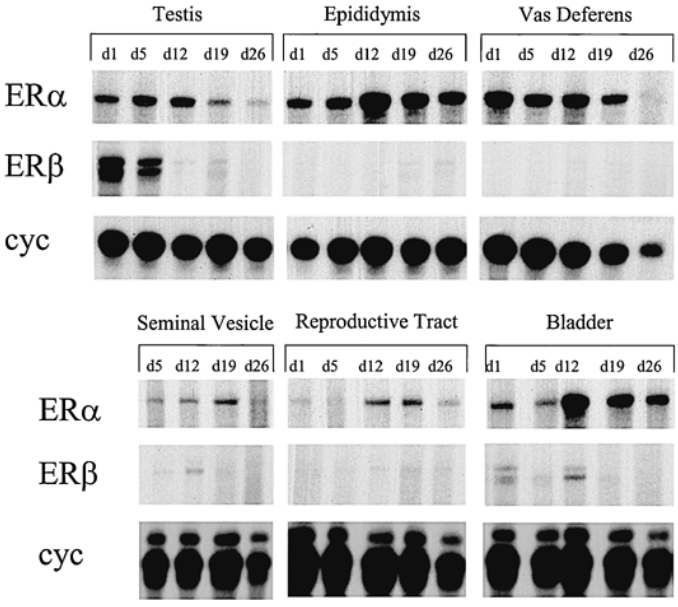

FIG. 3. RPA results for all postnatal male reproductive tract tissues. For each sample shown, 10 μg of total RNA was assayed. **Top**) Results for the testis, epididymis, and vas deferens. ERα mRNA was present in the testes, with decreasing mRNA levels with increasing age of the animal. ERα mRNA was also present in the epididymis and vas deferens at all ages examined, although at PND 26 an apparent decrease in mRNA in the vas deferens was seen. There was abundant ERβ mRNA in the PND 1 and PND 5 testis. ERβ was present at later time points; however, mRNA levels were relatively much lower. Although it is not apparent from this figure, ERβ was present in the epididymis and the vas deferens and can be more clearly seen in a longer exposure. **Bottom**) The results for the seminal vesicle, reproductive tract, and bladder. ERα and ERβ transcripts were detected in all samples assayed. Cyclophilin (cyc) demonstrates that the total RNA was comparable for all samples compared except PND 26 vas. The film was exposed overnight for ERα and ERβ and 8 h for cyclophilin.

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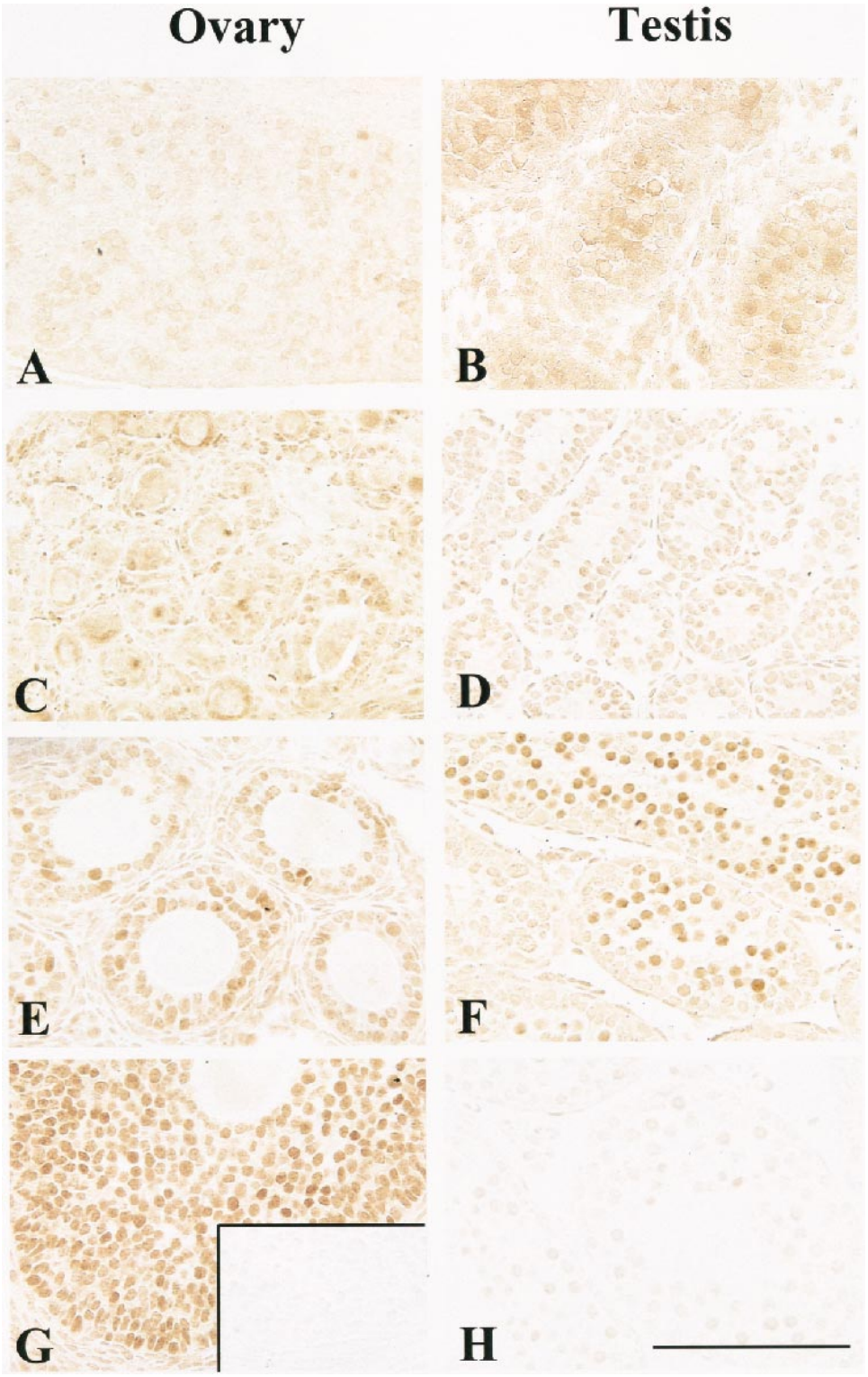

FIG. 4. Immunohistochemical analysis of ERβ in the developing ovary (left panels) and testis (right panels) at 16 dpc (**A** and **B**), and at PNDs 5 (**C,D**), 12 (**E,F**), and 26 (**G,H**). In the fetal ovary (**A**), there was no detectable ERβ immunoreactivity. ERβ immunoreactivity could be detected by PND 5 (**C**) and increased with the age of the animal, with the most abundant expression in the nucleus of the granulosa cells of the PND 26 ovary (**G**). ERβ immunoreactivity was detected in the testis at 16 dpc (**B**), PND 5 (**D**), and PND 12 testis (**F**), most predominantly in the spermatocytes on PND 12. There was little detection in the testis by PND 26 (**H**). The negative control for this immunostaining can be seen in the inset of **G.** The bar in **H** represents 100 μm, and all images are shown at the same magnification.

detectable ERα mRNA in the epididymis, vas deferens, seminal vesicle, reproductive tract (including the prostate), and bladder; varying levels of ERβ were seen in the same tissues (Fig. 3). Although not readily apparent in Figure 3, longer exposure times (2 days) revealed faint ERβ bands in the vas deferens and the epididymis (data not shown). The bladder also demonstrated the presence of ERα and ERβ mRNA early in development at varying levels for both transcripts.

Immunohistochemistry

Immunohistochemical localization of ERβ protein in the gonads during development is shown in Figure 4. The presence of ERβ as early as PND 5 in the ovary (C) followed the differentiation of granulosa cells, with the most abundant protein expression seen at PND 26 (G). The protein was localized to the nucleus of the granulosa cells of primary, secondary, and tertiary follicles. No immunostaining

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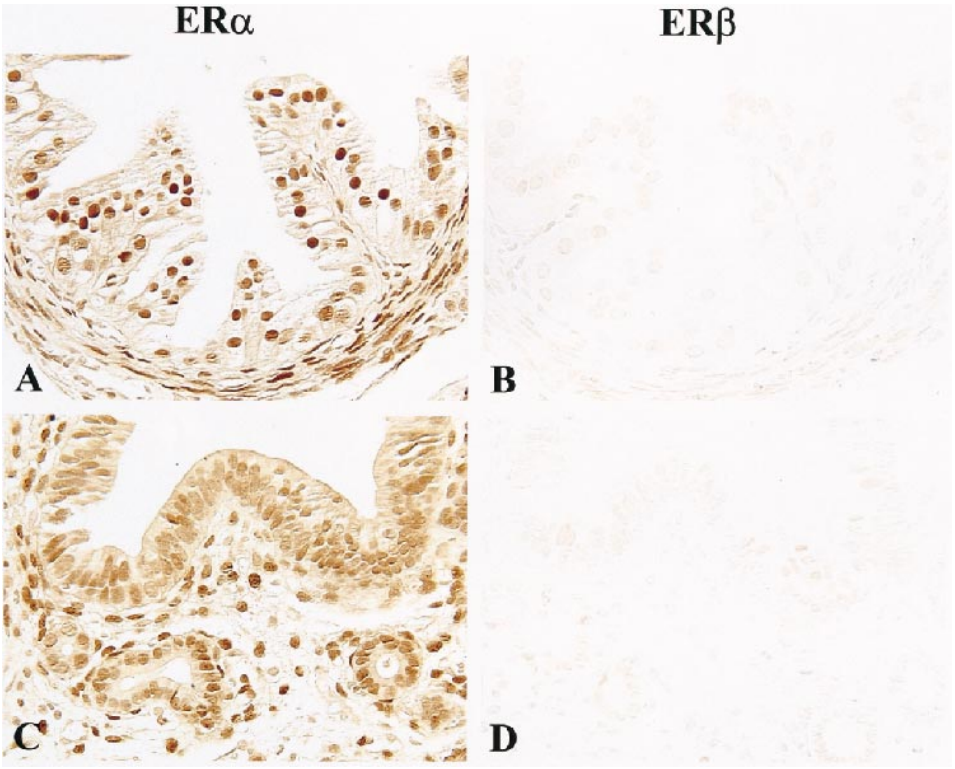

FIG. 5. Immunohistochemical analysis of ERα and ERβ in the PND 26 female reproductive tract. Intense ERα immunolocalization is seen in both epithelial and stromal cells of both the oviduct (**A**) and the uterus (**C**). However, ERβ immunostaining was not detected in either the oviduct (**B**) or the uterus (**D**).

of ERβ was seen in the interstitial cells of the ovary. Adjacent tissue sections immunostained for the presence of ERα exhibited specific staining in the interstitial cells of the ovary at all ages examined (data not shown). The presence of ERβ protein in the testis was observed in the germ cells as early as 16 dpc (B). The most abundant ERβ expression appeared at PND 12 in spermatocytes (F), after which the intensity decreased and was not apparent by PND 26 (H). Adjacent tissue sections immunostained for the presence of ERα indicated immunoreactivity in the interstitial cells of the testis at all ages examined (data not shown).

Immunostaining for both ER proteins was also determined in the female reproductive tract during development. ERα immunoreactivity was observed in all time points tested as previously reported [7]. ERα was detected in stromal cells of the uterus at all ages examined and in the epithelial cells as early as PND 5 and throughout the rest of development. There was no detectable expression of ERβ protein in the uterus at any time point examined even though levels of ERβ mRNA were seen at PND 1. An example of immunohistochemical localization of ERα and ERβ in the female reproductive tract can be seen in Figure 5. At PND 26, intense ERα immunoreactivity can be seen in the oviduct (A) and the uterus (C) in both epithelial and stromal cells. In contrast, ERβ immunoreactivity was not detectable in either the oviduct (B) or the uterus (D) in either cell type.

A comparison of ERα and ERβ immunoreactivity in the male reproductive tract at 12 days of age is shown in Figure 6. The epididymis exhibited strong ERα immunostaining in epithelial cells and light immunostaining in stromal cells (A); ERβ immunostaining was easily detectable in stromal cells and epithelial cells (B). The presence of both receptor forms in vas deferens was not as intense as in the epididymis; ERα was predominantly present in stromal cells (C), and ERβ was predominantly seen in epithelial cells (D). Seminal vesicles exhibited no detectable ERα immunoreactivity (E) although ERα mRNA was present in the RPA. ERβ immunoreactivity appeared in the epithelial cells of the seminal vesicle, but at a low level (F). The reproductive tract (seminal colliculus) exhibited immunostaining for ERα in the stromal cells (G) and for ERβ in the epithelial cells (H).

DISCUSSION

The ontogeny of ERα and ERβ was mapped in developing male and female mouse reproductive tracts. Fetal, neonatal, and immature female reproductive tract tissues exhibited an overall expression pattern of ERα and ERβ mRNAs that was similar to that reported in the adult [4]. In the ovary, ERα immunoreactivity was present in the interstitial cells, whereas ERβ immunoreactivity was seen in granulosa cells. ERβ levels in the ovary appeared to increase with age and the onset of follicular maturation, which involved increases in the granulosa cell population. An increase in cellular ERβ staining intensity indicated that the level of expression per cell was also increasing with age. The developing uterus showed an abundant amount of ERα in both stromal cells and epithelial cells but little or no expression of ERβ. Although a weak signal for ERβ mRNA was detected in the uterus in the neonatal mouse, specific immunoreactivity could not be detected by the methods used in this study. The general lack of detection of ERβ in the uterus is confirmed by the lack of function demonstrated by the uterine phenotypes described in ERα [28] and ERβ [29] knock-out mice.

Of particular significance was the presence of ERβ in the testis of fetal and neonatal mice, which was in contrast to the lack of expression we previously reported in adult mice [4]. In the current study, the developing testis contained ERβ immunoreactivity in the spermatocytes, but

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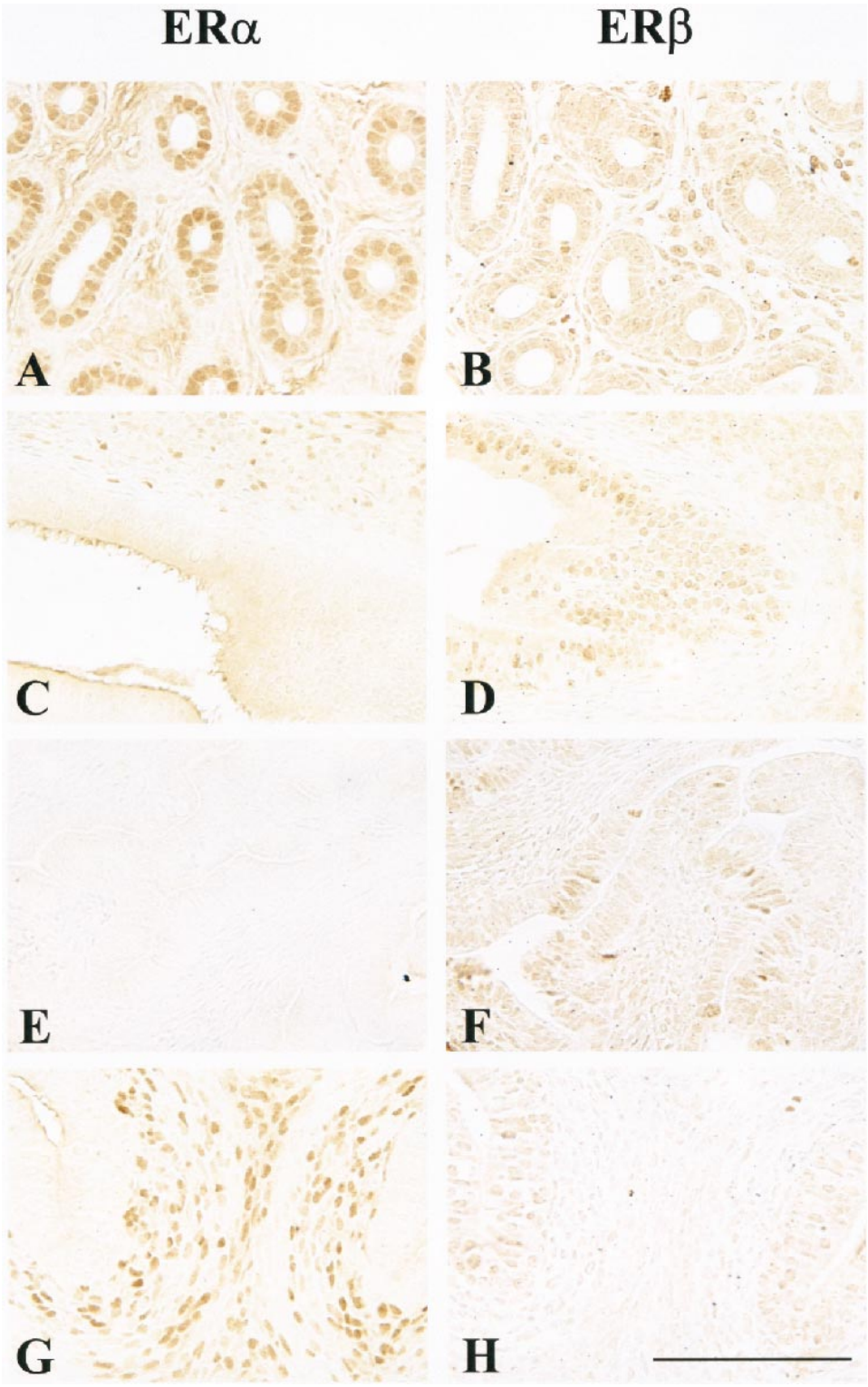

FIG. 6. Immunohistochemical analysis of ERα and ERβ in the PND 12 male reproductive tract. **A,B**) Epididymis, with localization of ERα predominantly in the epithelial cells with some protein in the stromal cells, and ERβ immunostaining in both stromal and epithelial cells. **C,D**) Vas deferens, with localization of ERα predominantly in the stromal cells and ERβ in the epithelial cells. **E,F**) Seminal vesicle, with ERβ protein found in the epithelial cells and no apparent ERα protein. **G,H**) Seminal colliculus, with ERα predominantly expressed in the stromal cells and ERβ showing a light immunostaining in the epithelial cells. The bar in **H** represents 100 μm, and all images are shown at the same magnification.

the immature testis showed no apparent ERβ immunoreactivity. ERα immunoreactivity was constitutively expressed in interstitial cells at all ages examined. By PND 26, the expression of ERβ in the testis diminished to undetectable levels when assayed by both RPA and immunohistochemistry. Thus, ERβ expression is apparently dependent on the stage of differentiation of the gonadocytes and disappears as the animal matures. Rosenfeld et al. [11] reported ERβ in Leydig cells of adult mice, a finding not consistent with our findings by either RPA or protein localization; however, they used a C57Bl/6J/129 mouse while we used an outbred CD-1 strain. Strain differences and different ages examined may account for the differences.

The relationship of ERβ message and protein mapped during development in the testis was interesting. The peak of ERβ mRNA occurred at PND 5 while the peak of immunoreactivity occurred at PND 12. Similar delays in translation of mRNA into protein have been reported for other transcripts found during oogenesis [30] and sper-

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matogenesis [31]. During spermatogenesis, cessation of transcription occurs within germ cells at certain stages of differentiation; apparently, it is necessary to synthesize and store mRNAs before their translation later during spermatogenesis [32]. For example, transcripts have been shown to be repressed during male germ cell differentiation by repressor proteins for several days before translation; protamine 1 mRNA was repressed by prm-1 RNA-binding protein [33]. Also, the developmental regulation of RNA binding proteins during spermatogenesis, like testis-brain RNA-binding protein, showed that mRNA translation could be regulated by proteins expressed during critical stages of development [34]. The mechanism of translational repression has been reviewed and is considered essential for normal spermatogenesis; premature translation of some of these mRNAs has been suggested to cause an arrest in spermatid differentiation resulting in male sterility [31]. The presence of ERβ in the neonatal mouse testis during critical periods of development could play an important role in normal differentiation. Any delay in the initial transcription or premature translation of the mRNA into protein could result in permanent alterations in the differentiation of the testis. Studies are currently underway to examine the mRNA levels as well as the protein expression of ERβ in the testis following neonatal exposure to exogenous estrogens.

The finding of ERβ only in spermatocytes of the neonatal mouse testis is in contrast to reports in the developing rat testis by Saunders et al. [18] and van Pelt et al. [35] showing ERβ in multiple cell types including Sertoli and peritubular cells. Both mice and rats, however, showed ERβ in spermatocytes in developing testis. The differences in mRNA and other protein cell types in the testis may be due to species differences, or to the antibody and procedures used for detection of the protein. The conflicting data in ERβ localization between the rat studies, specifically the differences seen in cell type distribution in the testis, are more striking than the differences between rat and mouse.

Tissues of the male reproductive tract exhibited expression of both receptor forms throughout development, similar to expression described in adults [4,9,10]. The developing male reproductive tract generally demonstrated the presence of both receptors in epithelial cells or stromal cells within a particular tissue. However, the seminal vesicle expressed ERβ immunoreactivity in epithelial cells but no detectable ERα immunoreactivity in either tissue compartment. This is in contrast to our RPA data that showed the presence of ERα in the seminal vesicles. Since ERα immunoreactivity was seen in the coagulating gland, ERα transcripts were probably contributed by this tissue, which was collected with the seminal vesicles for RNA analysis. Additional studies will be necessary to verify this possibility. The epididymis appeared to be the only tissue that shows the presence of both receptors colocalized in the same cell type. A gradient of ERα expression in the epithelial cells of the epididymis was apparent and agrees with that previously described at the RNA level [9,12,36]; highest expression was observed in the initial segment. ERβ expression also appeared to be higher in some areas of the epididymis than others. A more comprehensive study of the expression pattern within different segments of the epididymis is warranted to determine the specific receptor content throughout the epididymis. This gradient of expression of both receptors within the epididymis is not understood, but differential effects of estrogens through each receptor may be studied using this tissue. Effects demonstrated in one section or the other of the epididymis may be a key in understanding the mechanism of action of various exogenous estrogens on one receptor or the other.

The differences in tissue and cellular expression, as well as the appearance and disappearance of the two receptors, may play an important role in development and differentiation. Further, these differences may help explain the varied effects of exposure to different environmental estrogens at different stages of development. Specifically, the affinity of a compound for one receptor or the other may direct the adverse effects of that estrogen on certain tissues within the reproductive tract and be unique for a particular stage of developmental exposure. For example, since genistein preferentially binds to ERβ [25] and expression of ERβ is high in the ovary, this compound may cause changes in the ovary at doses lower than another estrogen that binds more efficiently to ERα. Studies are underway to test this hypothesis.

The presence of these two estrogen receptor subtypes in various tissues and their distribution in different cellular compartments adds further complexity to understanding of the role of estrogens in the developing animal. It is of interest to note that a recent publication [37] reported ERβ to be the dominant receptor subtype in the adult rat ovary and further localized it in the granulosa cells, which is in agreement with our findings in the developing mouse ovary. These investigators demonstrated that the ERβ protein was functional and was regulated by ovulatory doses of gonadotropins [37]. This suggests that the ERβ subtype most likely is a mediator of estrogen action in rodent granulosa cells during normal follicular development. The subfertility and ovarian dysfunction described in the ERβ knockout mice further suggests this hypothesis [29]. Therefore, the possibility now exists that ERβ may be involved in effects of various exogenous estrogens, particularly those that preferentially bind to ERβ. Experiments are underway in our laboratory to address this possibility. In summary, the data described in this report of normal developmental expression of the two receptor subtypes can be used as a map to further investigate abnormal differentiation of mice developmentally exposed to various environmental xenoestrogens.

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