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Differential Expression of Estrogen Receptor-β and Estrogen Receptor-α in the Rat Ovary

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ABSTRACT

Immunohistochemical localization of two estrogen receptor (ER) subtypes, ERβ and ERα, was performed in neonatal, early postnatal, immature, and adult rats to determine whether ERα and ERβ are differentially expressed in the ovary. ERβ and ERα were visualized using a polyclonal anti-ERβ antibody and a monoclonal ERα (ID5) antibody, respectively. Postfixed frozen sections and antigen-retrieved paraffin sections of the ovary revealed nuclear ERβ immunoreactivity (IR) in granulosa cells, which was prevented when peptide-adsorbed antibody was used instead. In immature and adult rat ovaries, ERβ was expressed exclusively in nuclei of granulosa cells of primary, secondary, and mature follicles. Atretic follicle granulosa cells showed only weak or no staining. No specific nuclear ERβ IR was detected in thecal cells, luteal cells, interstitial cells, germinal epithelium, or oocytes. In neonatal rat ovary, no ERβ expression was found. In ovaries of 5- and 10-day-old rats, weak ERβ IR was observed in granulosa cells of primary and secondary follicles, but no staining was detected in the primordial follicles. ERα protein exhibited a differential distribution in the ovary with no detectable expression in the granulosa cells but evidence of ERα IR in germinal epithelium, interstitial cells, and thecal cells. In the oviduct and uterus, IR for ERα, but not ERβ, was found in luminal epithelium, stromal cells, muscle cells, and gland cells. Our present study demonstrates that ERβ and ERα proteins are expressed in distinctly different cell types in the ovary. The exclusive presence of ERβ in granulosa cells implies that this specific new subtype of ERβ mediates some effects of estrogen action in the regulation of growth and maturation of ovarian follicles. (*Endocrinology* **140:** 963–971, 1999)

ESTROGEN influences the growth, differentiation, and functions of female and male reproductive tissues by acting through the estrogen receptors (ER) (1, 2), which are members of the superfamily of nuclear receptors (3, 4). Biochemical, autoradiographic, and immunocytochemical techniques have been used to detect ER in a variety of tissues (5–9). Biochemical and autoradiographic studies have previously demonstrated the presence of ER in brain, pituitary, and peripheral reproductive tissues, including the ovary and testis (10–14). Although specific binding of estradiol was found in granulosa cells (15–18) ER has not been localized to rat granulosa cells using receptor antibodies.

Recently, a novel ER complementary DNA (cDNA), designated and now known as the ERβ subtype, was cloned from the rat prostate (19) and mouse ovary (20). This cDNA is distinct from the classical ER cDNA (21), which is now recognized as the ERα subtype. The ERβ protein has highly conserved DNA- and ligand-binding domains compared with the ERα subtype (19, 22). The ERβ protein shares with the ERα protein about 95% homology in the DNA-binding domain and 55% homology in the C-terminal ligand-binding domain. Ligand binding assays have also shown that the ERβ protein binds estrogen with an affinity and specificity similar to those of the ERα protein (22). RT-PCR analysis and *in situ* hybridization revealed the highest levels of ERβ messenger RNA (mRNA) expression in the rat ovary and prostate (19, 22). Recent investigations also indicate the existence of several isoforms of rat ERβ mRNA (23, 24), one of which is known as ERβ2 and is expressed in the ovary, prostate, and other tissues (24). Rat ovary expresses both ERβ and ERα mRNA, but the ERβ mRNA is localized predominately in the granulosa cells of small, growing, and preovulatory follicles (25). However, limited information exists about the specific cellular localization of ERβ and ERα protein in rat ovary. The present study describes the differential distribution of ERβ and ERα in rat ovary, as visualized by differential immunocytochemical reactivity using a polyclonal antibody to synthetic ERβ peptide and a monoclonal antibody, ID5, which recognizes ERα, respectively. The results demonstrated that ERβ and ERα proteins were differentially expressed in rat ovary. ERβ was detected in granulosa cells, whereas ERα was localized in thecal cells, interstitial gland cells, and germinal epithelium.

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Materials and Methods

Animals

Timed pregnant Sprague-Dawley rats were purchased from Charles River Laboratories, Inc. (Raleigh, NC). The rats were fed NIH-07 rodent chow and water *ad libitum*. Neonatal (1 day old), postnatal (5–10 days old), immature (21–23 days old), and adult (60–70 days old) female offspring were used in the experiments. The animals were housed in humidity- and temperature-controlled rooms with a 12-h light, 12-h dark photoperiod. Neonatal and postnatal pups were kept with their dams until experimental use. The animal experiments were approved by the institutional animal care and use committee of the Chemical Industry Institute of Toxicology.

Tissue preparation

One-, 5-, and 10-day-old female rat pups were killed by decapitation, whereas immature and adult female rats were killed by CO_2 asphyxiation. The reproductive tract tissues, including ovary, oviduct, and uterus, were immediately removed and either frozen (see below) or fixed in buffered formalin for 6–24 h. Tissue sections were prepared from three to five rats in each age group.

For frozen sections, tissues were placed on Tissue-Tek OCT (Sakura Finetek, USA, Inc., Torrance, CA) mounts, frozen in isopentane precooled in liquid nitrogen (−180 C), and stored in a deep freezer (−80 C) until cryosectioning. Frozen sections of 8-μm thickness were cut and processed for immunocytochemistry. Fixed tissues were embedded in paraffin, and 5-μm sections were cut and processed for immunocytochemistry.

Histology

The classification of developmental stages of follicles were followed as previously described for the mouse and hamster ovaries (26, 27) and later adopted for the rat ovary (28).

Immunocytochemistry

The frozen sections were air-dried and then fixed for 5 min at room temperature in a mixture of 4% paraformaldehyde, 10% sucrose, and 0.1 M sodium phosphate buffer, pH 7.2. The paraffin sections were first deparaffinized and then treated with 3% H_2O_2 in PBS (pH 7.6) for 5 min. These steps were followed by heating the sections in a microwave oven (three times, 4 min each time) for antigen retrieval using a citrate buffer, pH 5.5–5.7 (1:10 dilution; HIER buffer, Ventana Medical Systems, Inc., Santa Barbara, CA). Postfixed frozen sections were treated with 3% H_2O_2 in PBS for 3 min to reduce endogenous peroxidase activity and then incubated with 0.2% Triton X-100 for 5 min. Postfixed frozen sections and antigen-retrieved paraffin sections were processed for immunostaining by the avidin-biotin peroxidase method as previously described (8). The sections were incubated overnight at 4 C with ERβ antibody (see below for details), preadsorbed ERβ antibody, and monoclonal antibody, ID5 (Dako Corp., Carpinteria, CA; see below for details). ERβ antibody was used at a concentration of 4 μg/ml in frozen sections and 10 μg/ml in paraffin sections. Monoclonal antibody was used at a concentration of 0.1–0.2 μg/ml. The optimal working dilution of antibody was determined by incubating sections with varying concentrations of antibody, ranging from 0.1–10 μg/ml. Sections were washed in 1 mM PBS (pH 7.6) followed by incubation with the secondary antibody goat antirabbit IgG or horse antimouse IgG and Elite avidin-biotin peroxidase at a concentration of either 1:100 or 1:200 for 30–60 min each at room temperature. After a 5-min wash, the sections were treated with liquid diaminobenzidine (Biogenex, San Ramon, CA) followed by a 10-min wash in PBS and then counterstained with hematoxylin. The immuostained slides were evaluated with an Olympus Corp. Vanox-S photomicroscope (Melville, NY). For comparison of immunostains, sections of ovaries from rats of different age groups were processed for immunostaining in parallel with the standard procedure described. The intensity of immunostaining was semiquantitatively designated as weak, medium, strong, or no staining.

Antibody

ERβ. A rabbit polyclonal antibody (PAI-310) raised against a synthetic peptide corresponding to the C-terminal amino acid residues 467–485 of rat ERβ was purchased from Affinity BioReagents, Inc. (Golden, CO). Characterization of this antibody by Western blot and gel supershift was limited to rat ERβ being overexpressed in COS-7 cells. Preadsorbed ERβ antibody was prepared by incubating 4–10 μg/ml ERβ antibody with 16–40 μg synthetic peptide for 24 h at 4 C.

ERα. Monoclonal antibody (clone 1D5; Dako Corp.) binds to ER and localizes ER in target tissues by both immunofluorescence and immunoperoxidase (29). In the present study, we compared immunostaining of the rat uterus using the monoclonal (Dako Corp.) antibody with that produced by a polyclonal antibody ER (MC20, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). MC-20 is an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to amino acids 580–599 mapping at the carboxyl-terminus of the ER of mouse origin.

Results

ERβ immunocytochemistry in female reproductive organs

In both frozen and paraffin sections of the ovary, nuclear ERβ immunoreactivity (IR) was observed in certain follicular cells, but not in others (Figs. 1, A, B, and C; 2A; and 3). The pattern of ERβ distribution appeared similar with the two types of tissue preparation, but the intensity of ERβ IR was stronger in frozen tissue sections (Fig. 1) than in paraffin-embedded tissue sections (Fig. 3). The specificity of the ERβ antibodies in the immunohistochemical reaction was ascertained by incubating adjacent tissue sections with the antibody preadsorbed with the synthetic peptide used as immunogen. Preadsorbed ERβ antibody did not reveal nuclear granulosa cell immunostaining in frozen sections (Fig. 1D) or in paraffin sections (Fig. 2B). Similarly, normal rabbit serum did not show nuclear immunoreaction (data not shown).

In ovaries of immature (21- to 23-day-old) rats, nuclear ERβ staining was observed in granulosa cells of the primary, secondary, and mature follicles (Figs. 1, A and B, and Fig. 3, A and B). The intensity of ERβ immunoreactivity was stronger in secondary and mature follicles than in primary follicles. Within the follicles, some granulosa cells did not show nuclear staining with ERβ antibody. Thecal cells, interstitial gland cells, oocytes, and germinal epithelium revealed a lack of nuclear ERβ immunoreactivity. However, interstitial gland cells showed cytoplasmic staining, which was not completely blocked but was reduced when preadsorbed ERβ antibody was used. In the uterus (data not shown) and oviduct (Fig. 1A), ERβ IR was not detected in luminal, stromal, gland, or muscle cells.

In both immature (21- to 23-day-old) and adult (60-day-old) female rats, nuclear ERβ expression was observed in granulosa cell nuclei of the growing follicles at all stages from primary to secondary and mature follicles (Fig. 3), including preantral and antral follicles. The intensity of ERβ immunostaining in granulosa cells of atretic follicles varied considerably. Some completely lacked IR, whereas in others, granulosa cells of the basal cell layers showed IR, and granulosa cells toward the center of the atretic follicle revealed no staining (data not shown). The atretic follicles were identified, as they consisted of granulosa cells with pyknotic nuclei and were clearly seen in adjacent ovarian sections stained with hematoxylin and eosin. Thecal cells and oocytes showed no nuclear staining; corpora lutea cells and interstitial gland cells had some cytoplasmic staining that was not completely blocked, as judged from sections incubated with preadsorbed ERβ.

ERβ expression in the developing reproductive organs

On postpartum day 1, histological examination of the ovary showed oocytes, differentiating stromal cells, and pregranulosa cells. Immunocytochemistry performed on sections of 1-day-old rat ovaries revealed no immunostaining with ERβ antibody in either oocytes or differentiating stromal cells and pregranulosa cells (Fig. 4A). From postpartum days 1–10, the ovary progressively increased in size; in ovaries from 5- and 10-day-old rats, primordial follicles, intermediate follicles, and growing follicles (primary and secondary) were recognizable (Fig. 4, B and C). At this age a small number of granulosa cells in the growing follicles showed nuclear ERβ staining (Fig. 4B), although the intensity of the IR appeared to be weaker than that in cells from immature and adult rat ovaries. No ERβ staining was de-

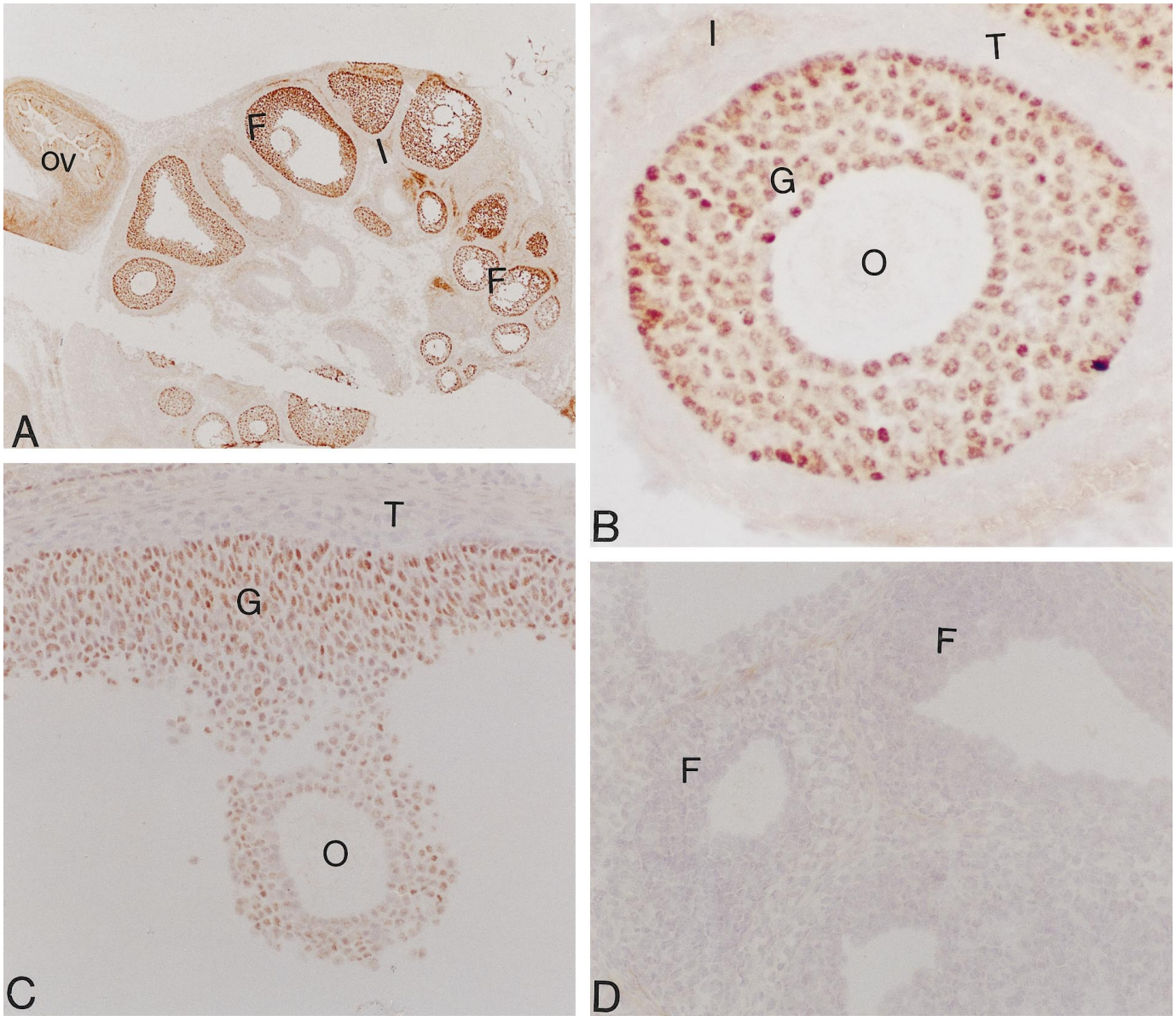

FIG. 1. Localization of ERβ in 23-day-old (A and B) and 60-day-old (C and D) rat ovary. Frozen sections of rat ovary were incubated with PAI P310 ERβ antibody (A–C) or preadsorbed ERβ antibody with the peptide immunogen (D). Note the immunostaining in follicles (F), specifically in nuclei of granulosa cells (G; B, C) and the lack of staining in thecal cells (T), interstitial cells (I), and oocytes (O). No staining was observed when peptide-adsorbed antibody was used (D). Counterstained with hematoxylin; magnification, ×110 (A), ×800 (B), and ×440 (C and D).

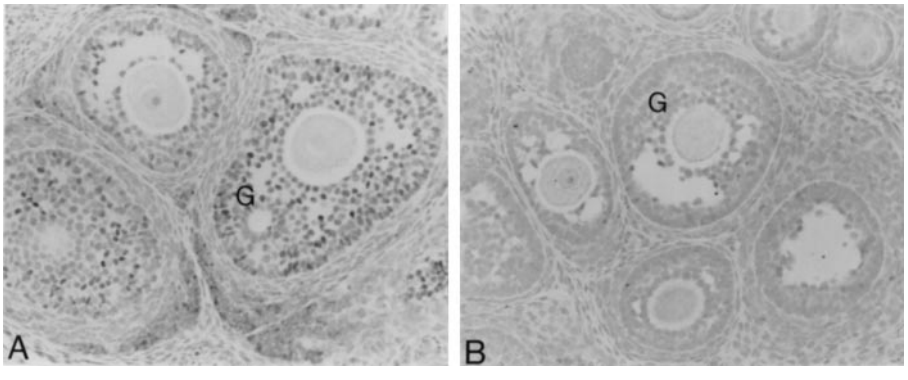

FIG. 2. Specificity of ERβ localization in 21-day-old rat ovary. Paraffin sections were stained with ERβ antibody (A) or ERβ antibody preadsorbed with the peptide immunogen (B). Note nuclear ERβ in granulosa cells (G), which was prevented when peptide-preadsorbed antibody was used. Counterstained with hematoxylin; magnification, ×295.

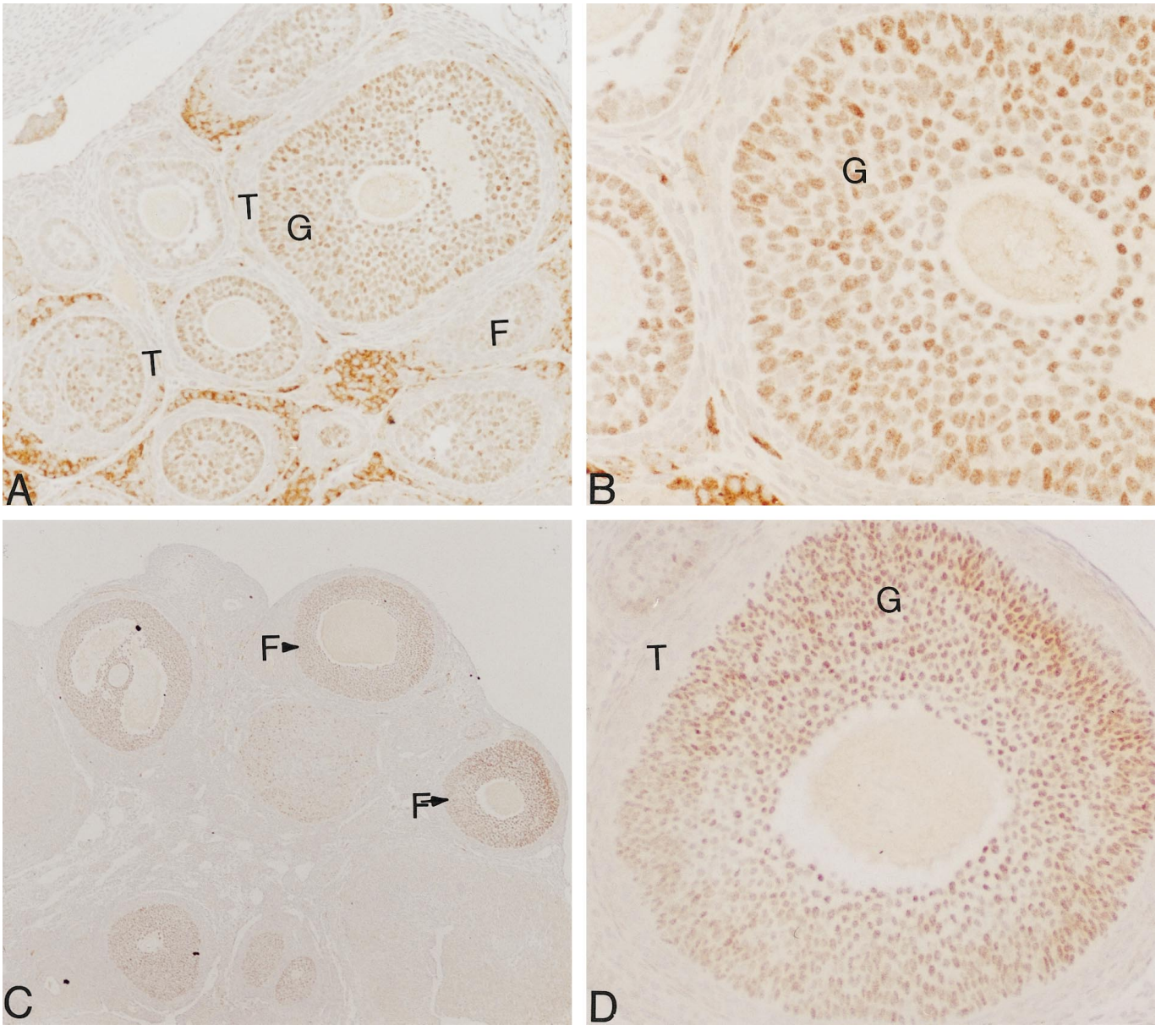

FIG. 3. Localization of ERβ in 21-day-old (A and B) and 60-day-old (C and D) rat ovary. Paraffin sections were immunostained with ERβ antibody after antigen retrieval using citrate buffer as described in *Materials and Methods*. Intense immunostaining was detected in follicles (F; primary, secondary, and mature) at different stages of development. Specific nuclear staining was observed in nuclei of granulosa cells (G). Some cytoplasmic staining could be seen in interstitial gland cells, but no staining was detectable in thecal cells (T). Counterstained with hematoxylin; magnification, ×360 (A and D), 725 (B), and 110 (C).

tected in primordial follicles, but very weak staining was seen in differentiating granulosa cells of the intermediate follicles (Fig. 4C). Oocytes, stromal cells, and germinal epithelium had no ERβ IR. Similarly, neither the uterus nor the oviduct of day 1 and day 10 rats revealed ERβ expression (data not shown).

To further compare the levels of ERβ expression in developing ovaries, we examined immunostaining intensities after incubation with two different concentrations (5 and 10 μg/ml) of ERβ antibody. At the 5 μg/ml concentration of ERβ antibody, no staining was detected in granulosa cells of 5-day-old rats, but weak staining was observed in 10-day-old rats, whereas a moderate or medium intensity staining was detected in immature and adult rats. At 10 μg/ml antibody, a strong intensity of IR was observed in immature and adult rat ovaries. Although ERβ expression was clearly seen in ovaries of 5- to 10-day-old rats, there were few positively stained granulosa cells, which revealed light to medium intensities of IR.

ERα immunocytochemistry in female rat reproductive organs

Immunocytochemistry with the ID5 monoclonal antibody revealed nuclear ERα expression in the uterus and oviduct of immature and adult rats (Fig. 5, A and F). The staining was

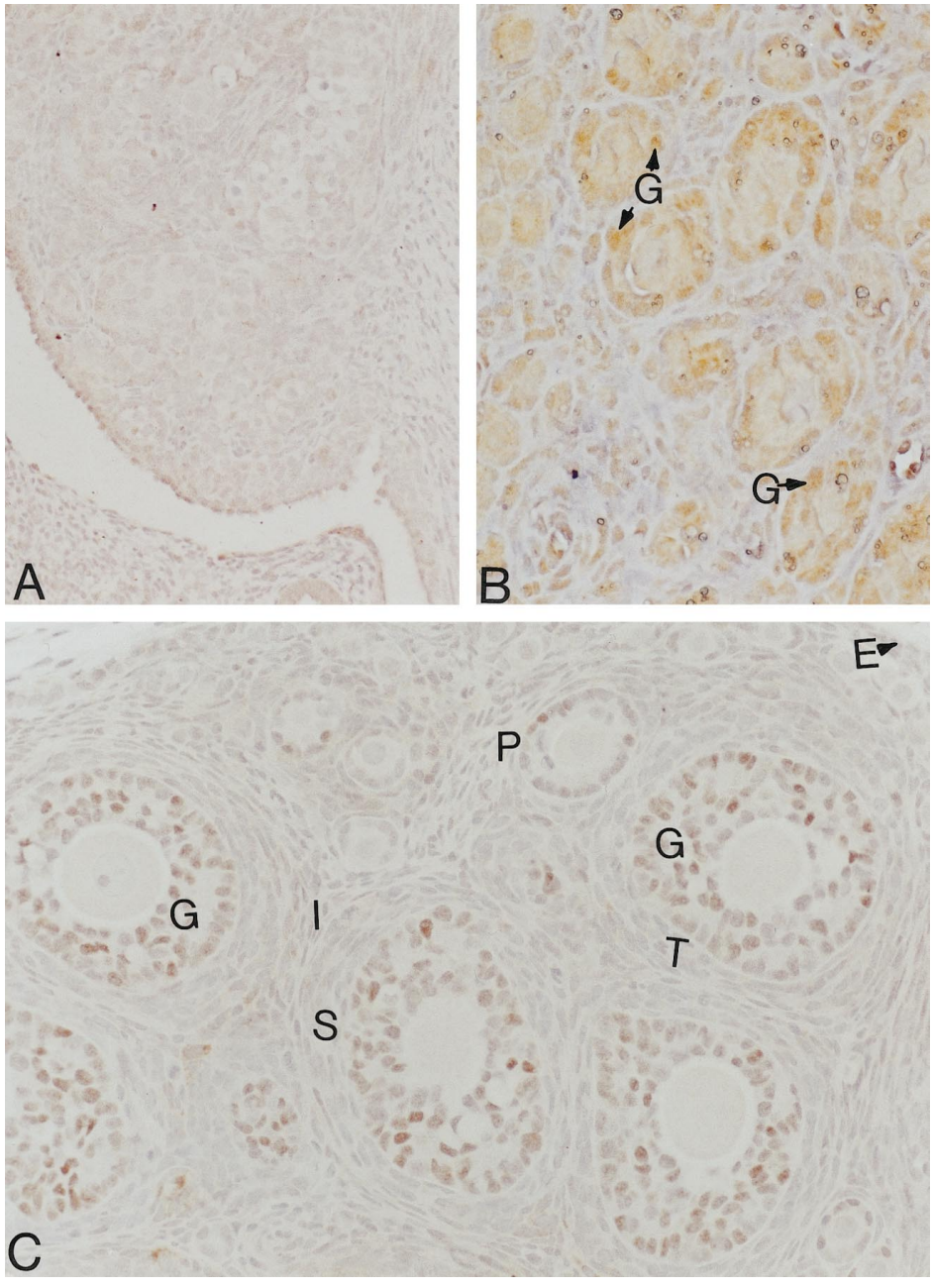

FIG. 4. Localization of ERβ in rat ovary on postnatal day 1 (A), day 5 (B), and day 10 (C). Paraffin sections were immunostained with ERβ antibody. A, In postnatal day 1 ovary, no staining was observed in cells of the undifferentiated follicles or the primordial follicles. B, On postnatal day 5 ovary, granulosa cells (G) of the primary follicles (intermediate and growing) showed weak to moderate IR. C, In postnatal day 10 ovaries, when follicles have grown in size, ERβ immunoreactivity showed increased intensity in granulosa cells (G). Note that the follicles (P, primary; S, secondary) at different stages of growth showed variable intensity of immunoreactivity. Germinal epithelium (E), thecal cells (T), and interstitial gland cells (I) did not show specific staining. Counterstained with hematoxylin; magnification, ×430 (A), ×800 (B), and ×730 (C).

specific, as adjacent sections of ovary and uterus did not show a positive immunoreaction when normal mouse IgG was used instead. A strong nuclear ERα IR was observed in luminal epithelial cells and gland cells as well as in stroma and muscle cells (Fig. 5F). The staining was also detected in the nuclei of these tissue sections, where the polyclonal MC-20 antirabbit ER antibody was used in place of the ID-5 monoclonal antibody (data not shown).

In the ovary of immature (21- to 23-day-old) rats, ERα nuclear staining was observed in thecal cells, interstitial gland cells, and germinal epithelium (Fig. 5, A–C). In contrast, no nuclear ERα IR was observed in granulosa cells of primary, secondary, and mature follicles. Similarly, granulosa cells of the same maturational stage follicles in adult rat ovary did not show nuclear ERα staining (Fig. 5, D and E). Corpora lutea cells also lacked nuclear staining with ERα antibody (Fig. 5D). In contrast, thecal cells, interstitial gland cells (Fig. 5, C–E), and germinal epithelial cells (Fig. 5, A and B) showed nuclear ERα staining. In both immature and adult rats, ERα staining was not detected in oocytes (O).

In neonatal rats, uterine stroma and muscle cells showed intense nuclear ERα staining, whereas luminal epithelial cells lacked IR (data not shown). In the oviduct, stromal cells, muscle cells, and luminal epithelial cells displayed strong nuclear staining. In the ovary, the germinal epithelium as well as differentiating stromal cells revealed specific nuclear ERα staining, whereas no reaction product was observed in pregranulosa cells and oocytes (Fig. 6A). In ovaries of 5- and 10-day-old rats, nuclear ERα staining was not detected in granulosa cells of primordial and growing follicles (Fig. 6,

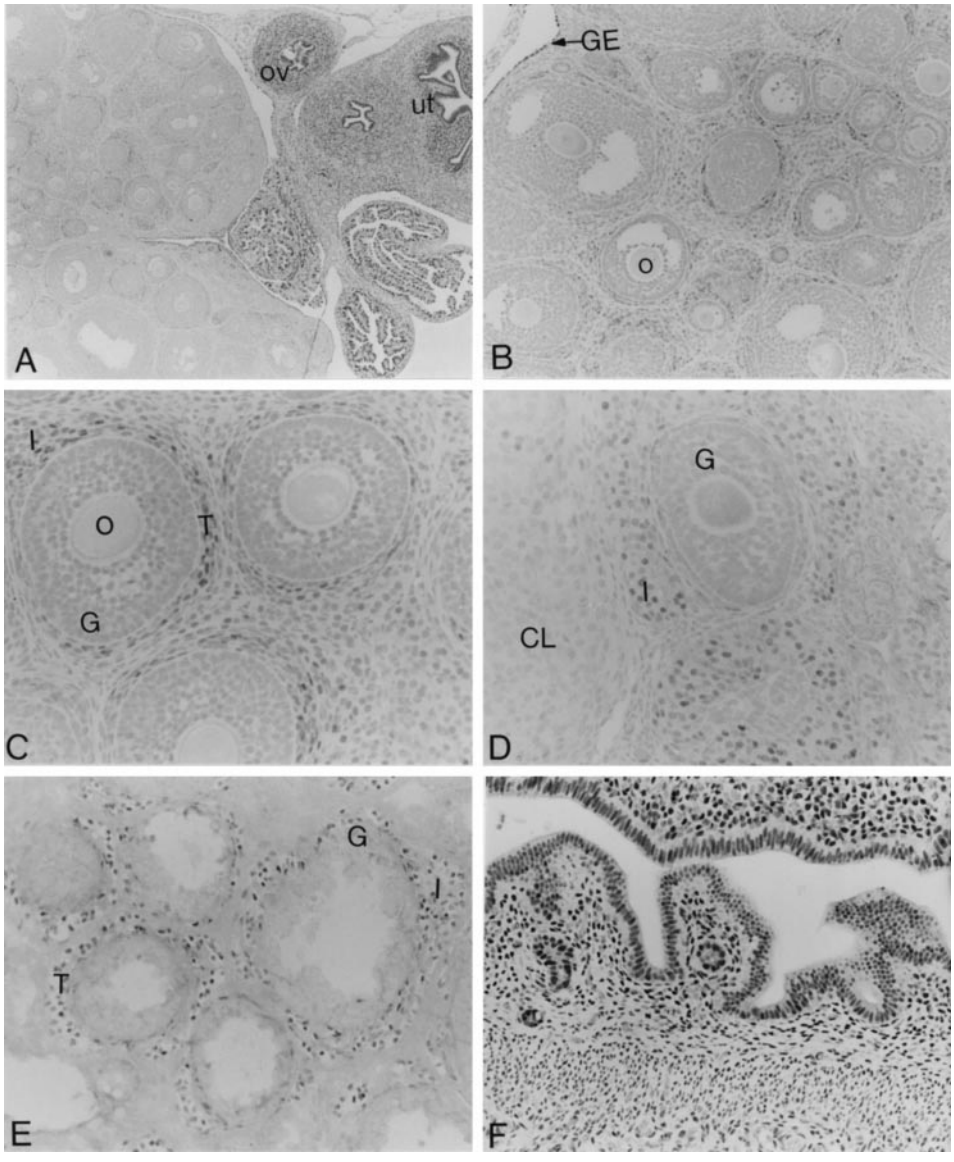

FIG. 5. Localization of ERα in ovary (A–E), oviduct (ov; A), and uterus (ut; A and F) of 21-day-old (A, B, C, and F) and 60-day-old (D and E) rats. Paraffin (A, B, C, D, and F) and frozen sections (E) were immunostained with a monoclonal antibody ID5. Intense ERα staining was detected in luminal epithelium and muscle cells of the oviduct (A) and uterus (A and F) as well as in stromal and gland cells of the uterus (A and F). Weak staining was observed in the ovary (A–E). In the ovary, no ERα was detected in granulosa cells (G) of primary, secondary, and mature follicles. In contrast, germinal epithelium (GE), thecal cells (T), and interstitial gland cells (I) showed nuclear ERα. Paraffin (D) and frozen (E) sections revealed similar staining. No ERα was detected in corpus luteum (CL) and oocytes (O). Counterstained with hematoxylin; magnification, ×70 (A), ×150 (B), and ×375 (C–F).

B–D). In contrast, ERα staining was seen in the nuclei of some stromal cells and germinal epithelial cells (Fig. 6, B and C), but weak staining was detected in a few thecal cells (Fig. 6D).

Discussion

The present study unequivocally demonstrates ERβ expression by immunocytochemistry in the rat ovary. Granulosa cells of primary, secondary, and mature follicles showed nuclear ERβ IR at different stages of follicular development. The immunodetection of ERβ in the rat ovary reported here is in general agreement with the nuclear localization of [^{3}H]estradiol in granulosa cells demonstrated previously both by autoradiography (13, 14) and by the binding of [^{3}H]estradiol to granulosa cells in biochemical assays (16, 30). Localization of the ERβ protein in granulosa cells also shows a parallel distribution with the high ERβ mRNA expression recently demonstrated by *in situ* hybridization and RT-PCR (19, 22) and agrees with a recent report describing ERβ localization to granulosa cells in the rat ovary (31). We also observed that the P310 ERβ antibody recognized immunoreactive ERβ in epithelial cells of rat prostate and epididymis (32, 33), in agreement with the expression of ERβ mRNA in these tissues reported previously (22).

In our immunocytochemical study, the distribution of ERβ protein differed from that of ERα in the rat ovary. ERβ was expressed in nuclei of granulosa cells but not in thecal cells or in interstitial gland cells and germinal epithelium. In contrast, ERα was localized in thecal cells, interstitial gland cells, and germinal epithelium, but not in granulosa cells. Immunoreactive ERβ was not detectable in either the uterus or the oviduct, although RT-PCR analysis has shown moderate expression of ERβ mRNA in the uterus. The lack of ERβ IR in the uterus may be attributable to low antigenicity of the primary antibody, which could not detect low levels of the ERβ protein. A recent report also indicates the presence of low levels of ERβ mRNA in the uterus and oviduct of mice (34). With the application of novel and more sensitive antibodies against ERβ protein, whether thecal cells, corpora

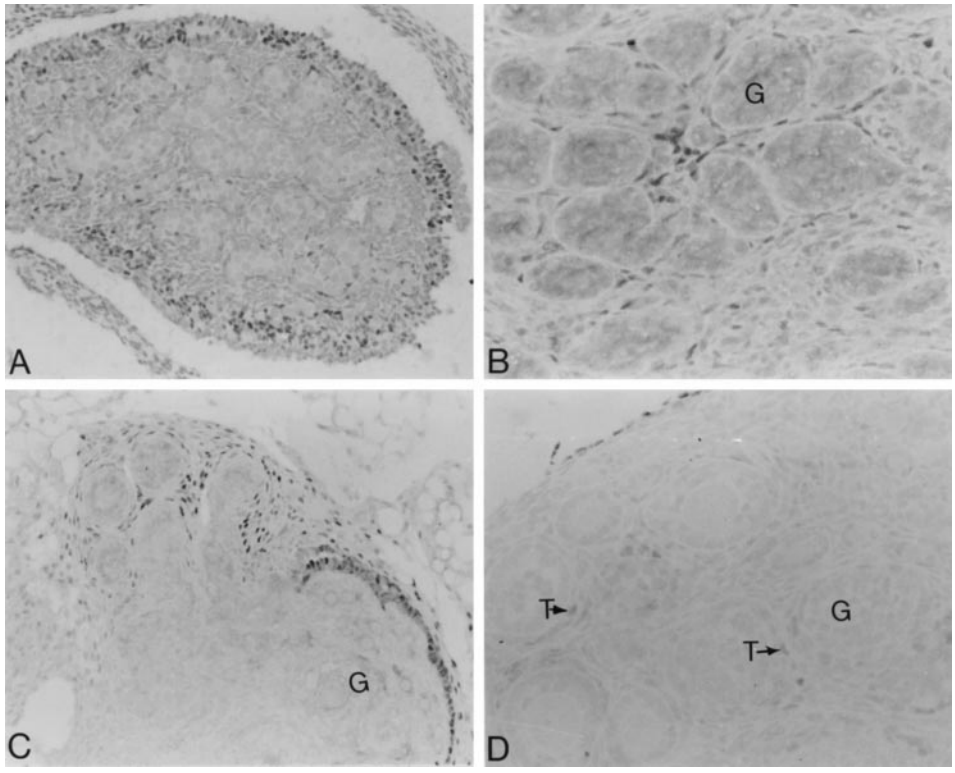

FIG. 6. Localization of ERα in rat ovary on postnatal day 1 (A), day 5 (B), and day 10 (C). Paraffin sections were stained with a monoclonal antibody. No staining was observed in granulosa cells (G) of primary and growing follicles on days 5 and 10. ERα was detected in undifferentiated stromal cells (A), connective tissue cells (A–C), and germinal epithelium (C and D). Weak IR was also seen in certain thecal cells (T), but not in others, in day 10 ovary (C). Counterstained with hematoxylin; magnification, ×450 (A and C) and ×600 (B and D).

lutea, and interstitial gland cells as well as uterine tissue express ERβ remains to be determined.

ERα and ERβ have similar binding affinities for estradiol (22), and nuclear localization of [^{3}H]estradiol has been demonstrated in granulosa cells, thecal cells, and interstitial gland cells (14). The coexistence of ligand-binding sites and ER in the ovary indicate that estrogen's effects on follicular cells and thecal cells are probably mediated through ERβ and ERα, respectively. The ERβ protein appears to be involved in follicular growth and maturation, as disruption of the ERα gene does not prevent the growth and maturation of small follicles (35), which express the ERβ, but not the ERα, protein. This interpretation is supported by recent findings that ERβ is down-regulated by gonadotropins in granulosa cells, suggesting the possibility of the physiological role of estrogen action in the ovary mediated by ERβ (25).

In the present study, we did not detect ERα receptor in oocytes using the ID5 monoclonal antibody. This observation does not exclude the possibility of a presence of α receptors in oocytes or an effect of estradiol on oocyte maturation. Recent data indicate that murine and human oocyte and cumulus-oocyte complexes express ERα transcripts, suggesting that a paracrine effect of estrogen is exerted on oocyte maturation (36, 37). Furthermore, transcription-independent or nongenomic effects of steroids, which presumably occur through plasma membrane receptors, have been reported (38–40). ERα message has also been detected in purified human granulosa cells, but the findings were not consistent (41). Similarly, ERα protein was not localized to granulosa cells, but has been detected in the granulosa cells of the antral follicles in primates and humans (42–45) and was found only in the P450 aromatase-containing granulosa cells of the antral follicle in humans (45). Specific binding of estradiol has been demonstrated in granulosa cells (43), and estradiol directly augumented stimulation of granulosa cell aromatase activity by FSH (46). A direct role of estradiol has been suggested in ovarian function in primates and humans (41). As estrogen binds to the ERβ protein with an affinity and specificity similar to those of ERα protein (22), a role of ERβ in ovarian tissue cannot be ruled out. On the basis of our findings of ERβ expression in rat ovary, a specific expression of ERβ protein can be expected in primate and human ovary.

The present data demonstrate the expression of ERβ IR in ovarian granulosa cells of growing follicles on postnatal day 5, whereas no ERβ IR was detected in the ovary on postnatal day 1, which only contains undifferentiated stromal cells and oocytes. In the postnatal day 5 ovary, weak immunoreactivity was observed in some granulosa cells. As follicles grew in size and granulosa cells proliferated to form a multiple layered epithelium, the numbers of ERβ-positive granulosa cells and their IR increased. An increase in staining intensity could be seen in growing follicles, which is clearly evident in the ovaries of 10- and 20-day-old rats. In contrast, expression of ERα protein was observed in stromal cells on postnatal days 1, 5, and 10; in thecal and interstitial cells of the 20-day-old ovaries; as well as in adult ovary. The developmental expression of ERβ indicates that the ERβ protein may be involved in follicular growth during postnatal development.

The expression of transcription factors GATA-4 and GATA-6 mRNA has been investigated in the mouse ovary during development and after hormonal stimulation (47). Abundant expression of GATA-4 mRNA has been detected in granulosa cells of primary and antral follicles, with lesser amounts in thecal cells, interstitial cells, and germinal epi-

thelium. Estrogen stimulation in immature mice has also been shown to increase the expression of GATA-4 and GATA-6 mRNA in granulosa cells (47). The results of this study indicated that GATA-4 and GATA-6 may play a role in the regulation of ovarian development, especially in the maturation and maintenance of follicles (47). As granulosa cells of primary and growing follicles express ERβ mRNA (19, 22) and ERβ protein, and the distribution of GATA-4 transcripts and ERβ protein overlaps in the granulosa cells of primary and growing follicles, ERβ protein probably interacts with the transcription factor GATA-4 in the regulation of granulosa cell function. Recent studies have indicated that members of the GATA-binding protein family form heterodimers with other GATA-binding proteins (48) and also form complexes with other classes of transcription factors (49, 50), including steroid hormone receptors (51). For example, estrogens exert effects on erythropoiesis by modulating GATA-1 activity through protein-protein interaction with the ER (48). Recent observations also indicate that ERβ can form a heterodimer complex and that a ERβ-ERα heterodimer is functionally active in subpopulations of target cells (52, 53). Thus, there is a possibility that ERβ may interact with GATA-4 through protein-protein interaction to induce some of the cellular functions in granulosa cells, as ERβ and GATA-4 are coexpressed in granulosa cells.

The present results indicate that ERβ was exclusively present in granulosa cells of primary and growing follicles, where it probably mediates some of the effects of estrogen action in the regulation of growth and development of the follicles. The differential expression of ERα and ERβ in specific cell populations of the rat ovary in conjunction with previously reported results of ERKO mice (35) suggests that both ER subtypes, ERα and ERβ, are essential for normal ovarian function. However, the biological function of the ERβ subtype is presently still unknown, and future studies of knockout mice with the disrupted ERβ gene function may provide information needed to understand the physiological action of ERβ.

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