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Progesterone Receptor Repression by Estrogens in Rat Uterine Epithelial Cells

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Measurements performed using cell lines or animal tissues have shown that the progesterone receptor (PR) can be induced by estrogens. By use of immunohistochemistry we studied the effects of estrogens on the PR levels in the individual cell types of the target organs uterus and breast. In the uteri of rats, ovariectomy induced a decrease in PR immunoreactivity within the myometrium and outer stromal cell layers. In contrast, in the uterine luminal and glandular epithelium and surrounding stromal cell layers the PR immunoreactivity was significantly enhanced. The same picture emerged when intact rats were treated with the pure estrogen receptor antagonist, ZM 182780 (10 mg/kg/d). Treatment of ovariectomized rats with estradiol resulted in high PR levels in the myometrium and stroma cells but low PR immunoreactivity in the epithelial cells. The ER-mediated repression of the PR immunoreactivity was evidently restricted to the uterine epithelium, as we found that in the epithelial cells of the mammary gland and in cells of N-nitrosomethylurea-induced mammary carcinomas the PR expression was induced by estrogens and was blocked by the pure antiestrogen ZM 182780. These results clearly show that in the rat the activated ER induces diverging effects on PR expression in different cell types even within the same organ. © **1997 Elsevier Science Ltd. All rights reserved**

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INTRODUCTION

The estrogen receptor (ER) is a nuclear transcription factor that — after activation by its ligand, estradiol (E2) — induces the expression of certain target genes [1, 2]. Estrogen receptor antagonists that bind to the ER and prevent its activation by the natural agonist have been described [3]. Pure antiestrogens such as ZM 182780 specifically and completely block the activity of the ER in many systems (= pure antiestrogens) [4].

One well known estrogen inducible target gene is the progesterone receptor (PR). From assays performed with whole tissue extracts the general picture emerged that PR levels are enhanced by estrogens and reduced by progestins [5–8]. Subsequent immunohistochemical studies revealed species and cell type specific differences in hormonal PR regulation (reviewed in [9]). In our study we compared the effect of selective ER blockade by a pure antiestrogen and ER activation by estradiol on the PR levels in different cell types of the rat. We provide evidence that in contrast to other cell types the PR is repressed by estrogens in the uterine epithelium.

MATERIALS AND METHODS

The rats were ovariectomized or treated with compounds for the period indicated. Analysis of the estrous cycle was done by vaginal smears. 17β-estradiol and ZM 182780 (7α-[9-(4,4,5,5,5,-pentafluoropentylsulfinyl)-nonyl]-estra-1,3,5,(10)-triene-3,17β-diol, synthesized in the laboratories of Schering AG, Berlin) were dissolved in castor oil containing 20% benzyl benzoate and administered 6 days per week by subcutaneous injection. At the end of the experiments the inguinal mammary glands and uteri were excised. The uteri were weighed and one half of the organ was directly snap-frozen in liquid nitrogen (for ligand binding assay). The other part of the organ as well

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Tek®, frozen in isopentane precooled with liquid nitrogen and stored at −80°C (for PR immunocytochemistry).

Measurement of PR from homogenates via ligand binding assay

For the extraction of whole PR the frozen tissues were pulverized and homogenized in high salt buffer (20 mM Tris, 10 mM Na_2MoO_4, 10% glycerol, 1.5 mM EDTA, and 400 mM KCl, pH 7.5) containing a protease cocktail. After centrifugation of the homogenates at 100 000 g (1 h, 4°C) and after determination of protein levels the extracts were diluted to a final KCl concentration of 50 mM for the ligand binding assay. This assay was performed using [^{3}H]-ORG-2058 with or without a 200-fold excess of unlabelled ORG-2058 to differentiate between unspecific and specific binding. Incubation was carried out at 4°C for 16 h. After separation of unbound steroid via the dextran-coated charcoal method the specific binding and thus the PR content was calculated as described [10].

Immunocytochemical detection of the PR in the rat

A minimum of three 5 μm cryosections of each specimen were performed. The sections were fixed for 10 min in 3.7% formaldehyde–PBS, 4 min in methanol (−20°C) and 2 min in acetone (−20°C). After blocking in rat serum (1:2 in PBS) for 30 min a monoclonal antibody against the PR (reacting with the PR-A and PR-B form, MA1-410, Dianova, Hamburg) [11] was applied at 20 μg/ml in PBS with 2% BSA for 18 h at 4°C. After blocking of endogenous peroxidases the second antibody-biotinylated rat anti-mouse IgG (Dianova) — was applied as a 1:600 dilution. The biotinylated secondary antibody was detected by the ABC technique (Vector) using diaminobenzidine. To check for unspecific binding all specimens were also incubated in parallel with a mouse IgG at 20 μg/ml instead of the first antibody. The resulting staining intensity was always very low. In addition, the specificity of the MA1-410 antibody was further verified by western-blot analysis of rat uterine extracts (not shown).

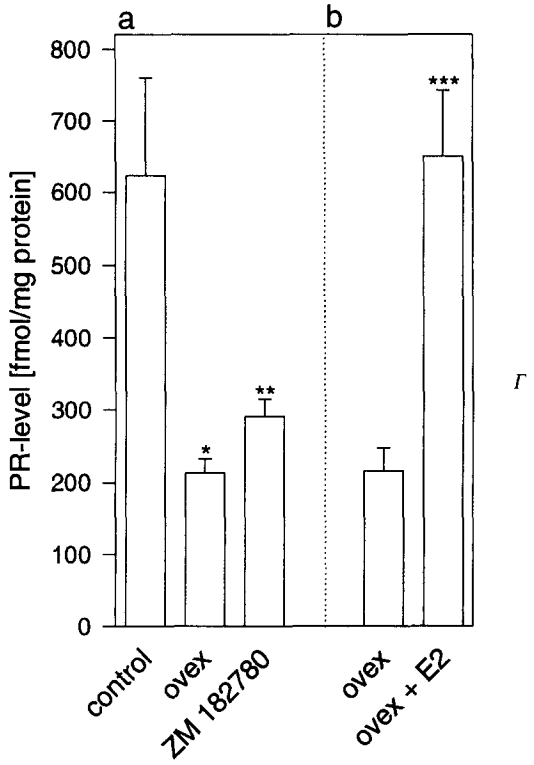

Fig. 1. Effect of ovariectomy or treatment with estrogens/antiestrogens on the PR level in homogenates of whole uteri: (a) Effect of ovariectomy or treatment of intact animals with ZM 182780 (10 mg/kg/d) for 21 days. (b) Treatment of ovariectomized animals with estradiol (0.1 μg/animal/d) for 28 days. The mean values ± S.E.M. are given. *$p < 0.01$ *vs.* control; **$p < 0.05$ *vs.* control; ***$p < 0.01$ *vs.* ovariectomy; Dunnet test ($n \geq 7$).

The intensity and distribution of specific staining were evaluated visually as described by Snijders *et al.* [12]. A receptor score was calculated as follows:

$$\text{Receptor score} = \sum_{i=0}^{i=4} P(i) \times i$$

where i is intensity of staining from 0 (no staining) to 4 (very intense staining) and $P(i)$ is the percentage of stained cells in category i (0–100%). From each specimen three cross sections were scored independently

Fig. 2 (facing page). PR immunoreactivity in the different uterine cell types of intact and ovariectomized rats.

(a,b) PR staining pattern in intact rats (estrous phase). (a) Myometrium: nuclei of the myometrial inner and outer smooth muscle cell layers are prominently stained. Nuclei of blood vessel cells are also PR positive. (b) Endometrium: The fibroblast-like stromal cell nuclei are PR positive. The luminal epithelium shows the typical increase in epithelial cell height. These cells show virtually no PR positive staining (arrow).

(c,d) PR staining 21 days after ovariectomy: (c) Myometrium: No or only marginal PR immunoreactivity is detectable. (d) Endometrium: High PR expression in the luminal and glandular epithelial cells (arrow) and in the surrounding stromal cell layers. Toward the outer stromal cell layers the PR expression is gradually reduced. The low epithelial cell height is typical for uterine histology after ovariectomy. Magnification: 200 ×; bar ≡ 50 μM. M = myometrium; S = stroma; E = epithelium.

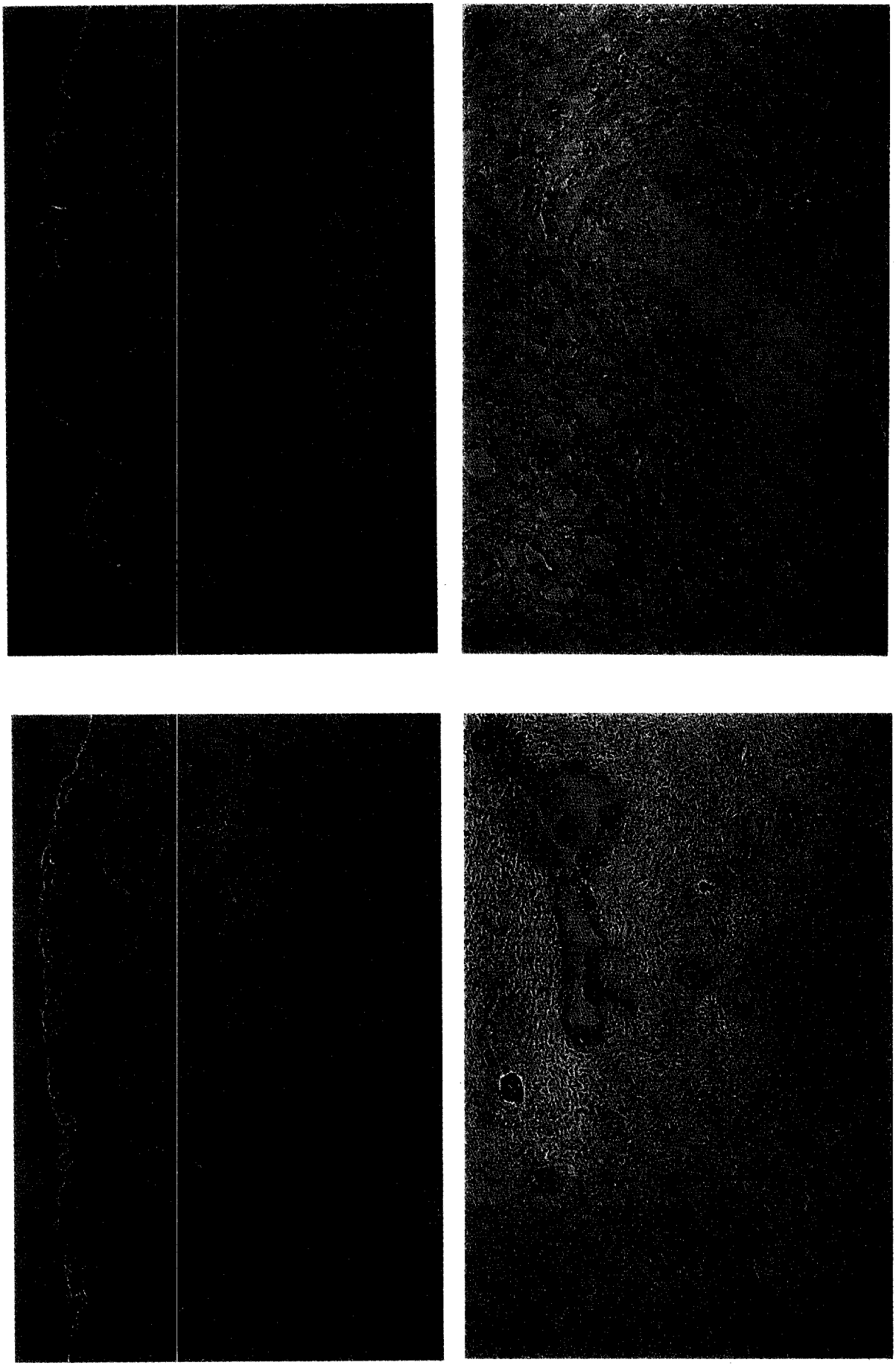

Figure 2—caption opposite

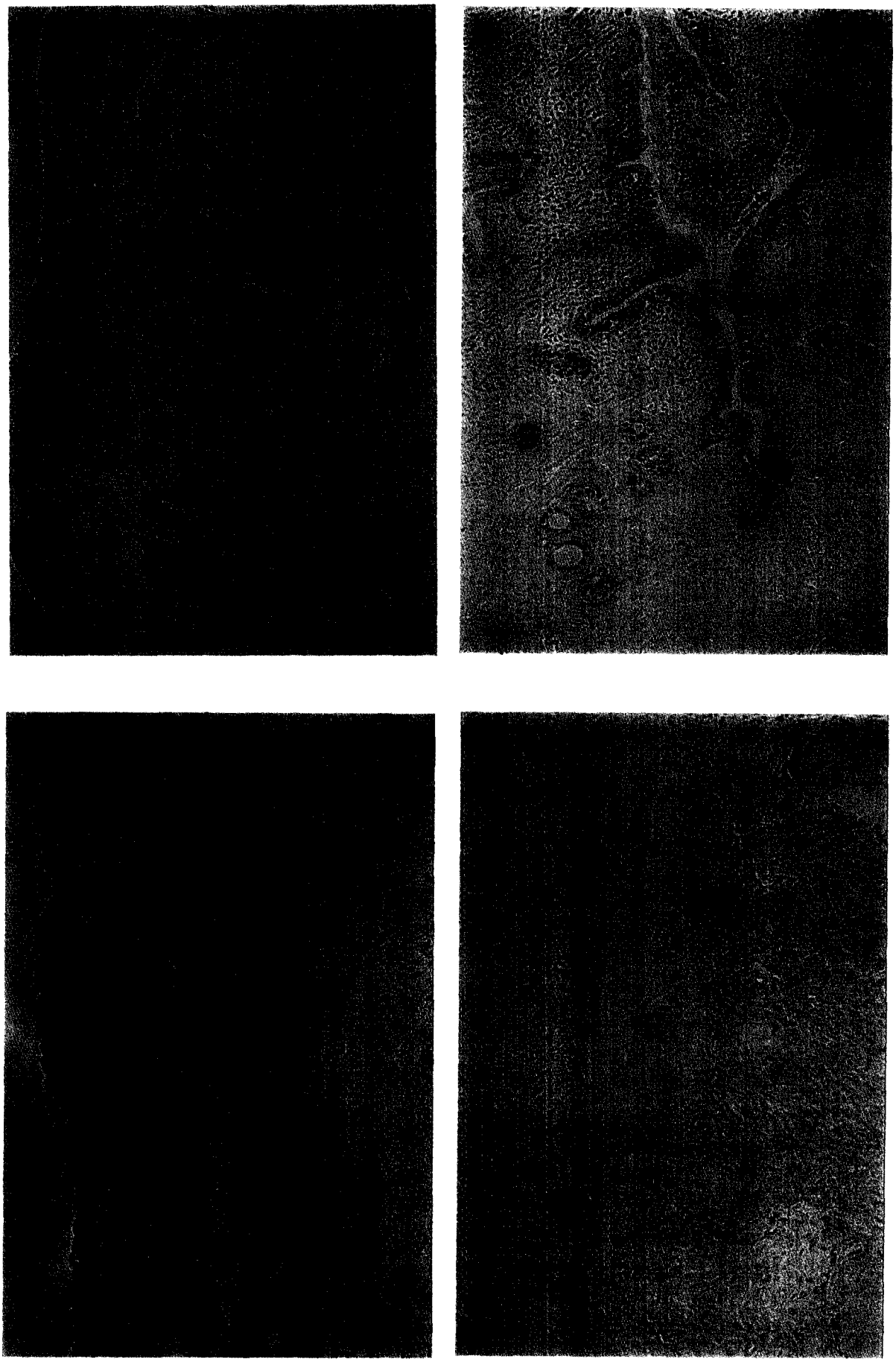

Figure 3—*caption opposite*

by two investigators. The scoring results of the individual investigators correlated with an r^2 value of 0.79.

RESULTS

Studies on the effects of ovariectomy, the antiestrogen ZM 182780 or treatment with estradiol (E2) on the PR expression in the rat uterus by means of the classical ligand binding assay using extracts of whole tissue homogenates revealed that the PR level started to decline 3 days after ovariectomy and after 14 days had reached 50% of the untreated controls (not shown). The PR levels had not declined any further 3 or 4 weeks post ovariectomy [Fig. 1(a)]. Treatment of intact animals with the pure antiestrogen ZM 182780 (10 mg/kg/d for 21 days) resulted in a PR level comparable to ovariectomy. When ovariectomized animals were treated with E2 (0.1 μg/animal/d for 28 days) the PR levels were restored to the level of intact animals [Fig. 1(b)].

The fact that the PR level declined by only 50% after ovariectomy or treatment with the pure antiestrogen ZM 182780 raised the question of whether PR expression in the uterus is, in general, not fully dependent on estrogens or whether the PR is differentially regulated by estrogens in the various uterine cell types.

The immunohistochemical analysis to study the cell type specific PR expression revealed that the PR staining was localized exclusively in the nuclei of all cell types looked at and after all treatment regimes carried out. In the uteri of intact rats changes in PR immunoreactivity during the estrous cycle were obvious. We found high PR expression in the myometrial smooth muscle cells, blood vessels and in stromal cells during the estrous (and proestrus) phase where the serum E2 levels were high [Fig. 2(a and b); Fig. 5 for quantification]. After ovariectomy, the PR expression significantly declined in the myometrium and in the outer stromal cells, whereas in the luminal and glandular epithelium and the surrounding cell layers the PR immunoreactivity was significantly enhanced [Fig. 2(c and d); Fig. 5].

The specific blockade of the ER function in intact animals by use of the pure ER antagonist (ZM 182780; 10 mg/kg for 21 days) also resulted in an ovariectomy-like PR expression pattern — significant PR repression in the outer uterine cell layers and PR induction in the uterine epithelium [Fig. 3(a and b); Fig. 5]. Treatment of ovariectomized animals with E2 (0.3 μg/animal/day for 14 days) resulted in a high PR expression in all cell layers except the uterine epithelium where its expression was repressed [Fig. 3(c and d); Fig. 5].

Our studies of the mammary gland revealed that the PR was exclusively expressed in the epithelial cells of the glandular buds (Fig. 4). In contrast to the uterine epithelium, the PR was fully down-regulated after ovariectomy or treatment with the pure antiestrogen ZM 182780 [Fig. 4(b and c); Fig. 5 for quantification]. E2 treatment (0.1 μg/animal/d) of ovariectomized animals again resulted in high PR immunoreactivity [Fig. 4(d); Fig. 5]. In accordance with this, we found in homogenates of *N*-nitrosomethylurea (NMU)-induced mammary carcinomas of the rat that after treatment with ZM 182780 (25 mg/kg/d) the PR was reduced nearly to the limit of detection (not shown).

DISCUSSION

In this study, we describe the effects of ovariectomy, treatment with estradiol (E2) or a pure antiestrogen on the PR immunoreactivity pattern in the different cell types of the uterus and mammary gland. All studies were carried out with adult rats and long term treatment was performed to look at the effects when steady-state levels are achieved.

Fig. 3 (facing page). Impact of the antiestrogen ZM 182780 or estradiol on the PR immunoreactivity in the rat uterus.

(a,b) PR distribution in the uterus of intact animals treated with the pure antiestrogen ZM 182780 (10 mg/kg/d for 21 days). (a) Myometrium: Nuclei of smooth muscle and blood vessel cells are only weakly stained. (b) Endometrium: High PR staining intensity in the luminal and glandular epithelial cells (arrow). The adjacent stromal cells are also PR positive. The PR expression declines towards the outer stromal cell layers.

(c,d) PR staining of ovariectomized animals treated with E2 (0.3 μg/animal/day for 14 days): (c) myometrium, (d) endometrium: The PR staining pattern and intensity is similar to intact animals in proestrus or estrus [Fig. 2(a, b)]. The nuclei are deeply stained in the smooth muscle and stromal cells. The epithelial PR immunoreactivity is low (arrow). Magnification: 200 ×; bar ≡ 50 μM. M = myometrium; S = stroma; E = epithelium.

Fig. 4 (overleaf). Effect of ovariectomy and/or treatment with estradiol or the antiestrogen ZM 182780 on PR expression in the mammary gland: (a) intact control; (b) ovariectomy; (c) intact animals treated with ZM 182780 (10 mg/kg/d) for 21 days; (d) ovariectomy and treatment with estradiol (0.1 μg/animal/d for 28 days). Magnification: 200 ×; bar = 50 μM.

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