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Down-regulation of androgen receptor by progestins and interference with estrogenic or androgenic stimulation of mammary carcinoma cell growth *

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Summary. The regulatory influence of medroxyprogesterone acetate (MPA) on estrogen and androgen receptors of the human breast cancer cell lines MCF-7 and EFM-19 was explored in conjunction with the growth-promoting properties of these steroids. In the absence of steroidal stimulation, up to 1 μM MPA had no effect on the proliferation of the MCF-7 cell strain used and of EFM-19 cells. Under stimulation with 10 nM 17β-estradiol or 1 μM dihydrotestosterone, dose-dependent inhibition of the cell proliferation rates by 0.1–1 μM MPA was observed. Binding of MPA to the androgen receptor ($K_d = 2.1$ nM) but not to the estrogen receptor was demonstrable. During incubation of MCF-7 or EFM-19 cells with 1 μM MPA for 7 days, the estrogen and androgen receptor contents were down-regulated by approximately 50% and 60%, respectively. Likewise, the number of androgen-binding sites was reduced to 35% of the untreated controls after incubation of MCF-7 cells with 1 μM synthetic progestin R5020 for 7 days. The results indicate down-regulation of estrogen and androgen receptors by progestins in the absence of stimulatory effects on the proliferation of mammary carcinoma cells.

Key words: Androgen – Breast cancer – Cell culture – Estrogen – Progestins

Introduction

Different mechanisms have been proposed to explain the therapeutic efficacy of systemic breast cancer treatment with high-dose medroxyprogesterone acetate (MPA) (Blossey et al. 1984; Becher et al. 1989). Indirect inhibition of tumor growth may be achieved by interference of MPA with the endocrine-regulated host/tumor balance. Dose-dependent reduction of follicle-stimulating hormone and luteinizing hormone secretion followed by decrease of ovarian estrogen synthesis is induced by MPA treatment in premenopausal patients (Schulz et al. 1987). Suppression of the hypothalamo-pituitary-adrenal axis, as determined by the decrease of the serum levels of cortisol, was shown in therapy regimens in which weekly doses of 400–1200 mg MPA were applied (Hellman et al. 1976). In postmenopausal patients, successful therapy with MPA appears to be correlated with the degree of adrenal suppression indicated by the reduced levels of glucocorticoids (Wander et al. 1983). This is in accordance with earlier observations emphasizing the importance of adrenal steroids in the control of breast cancer, as demonstrated by the inhibition of tumor growth after adrenalectomy (Huggins and Bergenstal 1952). In postmenopausal women, adrenal androgens may serve as precursors for the peripheral aromatization to estrogens (Grodin et al. 1972; Santen et al. 1986), and their suppression by high-dose MPA treatment may contribute indirectly to the reduction of tumor growth.

Direct positive effects of steroid hormones on the growth of breast cancer cells were shown by in vitro investigations. In a number of human mammary carcinoma cell lines, the proliferation was stimulated by estrogens as well as by androgens (Lippman et al. 1976a,b; Simon et al. 1984a; Hackenberg et al. 1988). In cultures of MCF-7 cells both types of steroid were able to induce synthesis of the progesterone receptor (Zava and McGuire 1978), of which only low levels are found in unstimulated cells (Horwitz et al. 1975). During incubation with 10 nM MPA, [^{3}H]thymidine incorporation into the DNA of unstimulated MCF-7 cells decreased under serum-free conditions (DiMarco 1980). The proliferation of MCF-7 cells was accordingly inhibited by 1–10 nM MPA in culture medium with 4.7% fetal calf serum that had been freed of steroids by treatment with dextran-

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Abbreviations: MPA, medroxyprogesterone acetate; R1881, methyltrienolone-17β-hydroxy-17α-methylestra-4,9,11-triene-3-one; R5020, 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione; PBS, phosphate-buffered saline

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coated charcoal (Braunsberg et al. 1987). MPA had antiestrogenic properties in this cellular model system, and reduced the [^{3}H]thymidine incorporation in MCF-7 cells treated with the stimulatory concentration of 10 nM 17β-estradiol (E_2) to low levels, corresponding to that of control cultures kept in 10 nM MPA only. The antiestrogenic effect of MPA may not be based on competition at the receptor level, since MPA does not bind significantly to the estrogen receptor (Horwitz et al. 1975; Young et al. 1980). However, during incubation with MPA for 48 h, the estrogen-binding sites of MCF-7 cells decreased by 75% (DiMarco 1980). Down-regulation of estrogen binding in MCF-7 cells was explained recently by the reduction of estrogen receptor mRNA during incubation with the synthetic progestin R5020 (Read et al. 1989).

The in vitro effects of estrogens and androgens are similar with respect to the enhancement of proliferation and the induction of the progesterone receptor in MCF-7 cells (Lippman et al. 1976a,b; Zava and McGuire 1978). Quantitatively, androgens are less potent in MCF-7 cells, since 1 µM 5α-dihydrotestosterone was shown to be equivalent to 10 nM 17β-estradiol (Zava and McGuire 1978). In MCF-7 and EFM-19 cell cultures androgenic stimulation of the proliferation was inhibited by the antiandrogenic compounds cyproterone acetate and hydroxyflutamide (Hackenberg et al. 1988), supporting earlier reports on the involvement of the androgen receptor in growth promotion by dihydrotestosterone (Lippman et al. 1976b). Progestins will compete with the androgen binding of MCF-7 cells (Horwitz et al. 1975), although details on the androgenic or antiandrogenic properties of progestins are lacking. The present study was aimed at obtaining further insight in the interaction of MPA, especially during high-dose application, with the growth-stimulatory hormones 17β-estradiol and dihydrotestosterone in regard to regulation of the steroid receptor content of MCF-7 and EFM-19 mammary carcinoma cells.

Materials and methods

Origin of tumor cells. MCF-7 cells (Soule et al. 1973) were kindly provided by Dr. G. Daxenbichler, Universitäts-Frauenklinik, Innsbruck, Austria, EFM-19 (Simon et al. 1984a,b) and MFM-21 (Hackenberg et al. 1988) cells were established as permanent lines from the pleural effusions of patients with recurrent mammary carcinoma.

Cell cultivation. The monolayer stock cultures were grown in plastic culture flasks (Nunc, Roskilde, Denmark) in a humidified atmosphere with 5% CO_2. The growth medium consisted of Earle's salts with phenol red, Eagle's minimal essential medium amino acids and vitamins (Biochrom, Berlin, FRG). The pH value was adjusted to 7.2 by the addition of $NaHCO_3$. The growth medium was enriched with 4 mM L-glutamine, 1 mM sodium pyruvate (Biochrom), 5 mg/l fetuin, 2.5 mg/l transferrin, 250 nM glycyl-L-histidyl-L-lysine, 10 nM triiodothyronine, 0.1 nM 17β-estradiol (all from Serva, Heidelberg, FRG), 40 IU/l insulin (Hoechst, Frankfurt, FRG) and 10% fetal calf serum (Boehringer, Mannheim, FRG). The medium of the cultures was renewed every 3–4 days. At confluence the cell cultures were subcultivated with 0.05% trypsin/5 mM EDTA (Serva) in phosphate-buffered saline (PBS: 0.14 M NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4; pH 7.2).

Experimental growth conditions. Reduced medium for the experiments consisted of the same components as growth medium with the exception of hormones and growth factors. In order to remove endogenous steroids, fetal calf serum was treated twice for 30 min at 55° C with 0.5% charcoal (Norit A, Serva) coated by previously mixing with 0.05% dextran T70 (Pharmacia, Uppsala, Sweden) (Darbre et al. 1983).

Proliferation assay. After detachment from stock cultures by trypsinization, the cells were suspended in growth medium at a concentration of 10^4 cells/ml, and 1 ml of this suspension was seeded in multiple wells of 4 × cluster dishes (2 cm^2, Nunc). After the attachment phase of 1 day, the cultures were rinsed with PBS and supplied with 1 ml reduced medium. The steroids under investigation were dissolved in absolute ethanol and further diluted with PBS. Samples of 25 µl in tenfold dilution steps were added to each culture dish to obtain the hormone concentrations desired. The final ethanol concentration was less than 0.1% and had no influence on the proliferation rates. Medium and hormones were renewed after 2 and 4 days. Estradiol-17β and dihydrotestosterone were purchased from Serva (Heidelberg, FRG). Medroxyprogesterone acetate was a gift from Upjohn (Heppenheim, FRG). R5020 was obtained from NEN (Frankfurt-Dreieichenhain). After the incubation period of 7 days the cells were detached from the dishes by trypsinization and counted in a hemocytometer.

Binding studies with [^{3}H]estradiol and [^{3}H]R1881. Samples of 5 × 10^4 cells were seeded per culture dish. Reduced medium was applied after the attachment period of 1 day. The cells were grown for 7 days with or without the indicated concentrations of MPA. Prior to the receptor assay, the cell layers were rinsed three times with PBS to remove free MPA. Thereafter, 17β-[^{3}H]estradiol (sp. act. 42.4 Ci/mmol) or the synthetic androgen [^{3}H]R1881 (sp. act. 86 Ci/mmol) (NEN) was added in the range of 25 pM to 2.5 nM with or without a 100-fold concentration of the appropriate unlabeled compound. The androgen receptor assays with [^{3}H]R1881 were performed using a 1500-fold excess of triamcinolone acetonide (Sigma, Munich, FRG) to block binding of the labeled androgen to the glucocorticoid receptor. After an incubation period of 4 h at 37° C the culture dishes were rinsed twice with PBS, and 0.5 ml 1 M NaOH was added to solubilize the cells. The samples were neutralized with 1 M HCl, and the radioactivity was determined in a β-scintillation counter using 10 ml Scinti-G (Roth, Karlsruhe, FRG). The data were analyzed as Scatchard plots by linear regression (program Lotus 123, Lotus Development Corporation, Berkshire, UK). Binding of MPA to the receptors for estrogen and androgen was investigated by incubation of cell cultures with 1 nM–1 µM unlabeled MPA in addition to the radioactively labeled steroids. The affinity of unlabeled MPA was calculated indirectly from the displacement of 17β-[^{3}H]estradiol or [^{3}H]R1881 from the receptors by an approach for competitive binding (Dixon and Webb 1971; Hofmann and Sernetz 1983). The K_d of MPA was equivalent to $K_1 \times c/(K_2 - K_1)$, in which c was the concentration of the unlabeled substance, K_1 was the K_d of the labeled compound, and K_2 was the apparent reduced K_d of the labeled compound in the presence of MPA.

Results

Effects of MPA on cell growth

The interaction of MPA with estrogenic or androgenic stimulation of the proliferation was investigated with cells of the permanent lines MCF-7 and EFM-19. In 7-day experimental cultivations and in the absence of stimulatory concentrations of 17β-estradiol, MCF-7 cells incubated with MPA in the concentration range of 1 nM

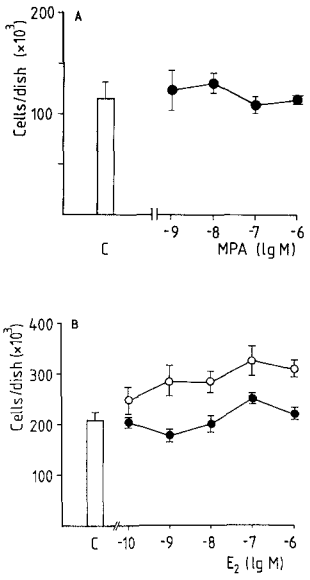

Fig. 1A,B. Proliferation rates of MCF-7 cells in the presence of MPA during 7-day cultivation periods. **A** Under estrogen-free conditions, lack of growth-inhibitory effect of MPA in the concentration range of 1 nM to 1 μM. **B** Stimulation of the cell proliferation by 0.1 nM to 1 μM 17β-estradiol (E_2) (○) and reduction of the estrogenic stimulation by the simultaneous application of 10 nM MPA (●). The cell growth in untreated control cultures is indicated by the *open bars*. Before application of the hormones, the cell numbers per dish were 7.8×10^3 (**A**) and 12.5×10^3 (**B**). The values represent mean cell counts ± SD of quadruplicate cultures

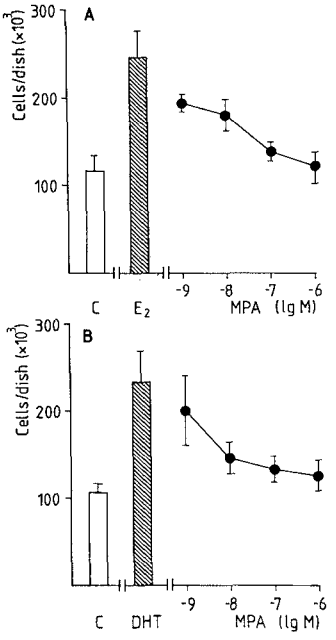

Fig. 2A,B. Dose-dependent interference of MPA with estrogen- or androgen-stimulated proliferation rates of MCF-7 cells. The *shaded bars* indicate the increase of cell growth induced by the sole application of 10 nM 17β-estradiol (E_2) (**A**) or 1 μM dihydrotestosterone (*DHT*) (**B**). The proliferation rates of the corresponding untreated control cultures are represented by the *open bars*. Before hormone administration, the cell numbers per dish were 7.7×10^3 (**A**) and 6×10^3 (**B**). The results are expressed as means ± SD of quadruplicate cultures

to 1 μM showed the same proliferation rates as the control cell cultures (Fig. 1A). Compared with the onset of experimental incubation, the cell numbers had increased by a factor of approximately 14 at the termination of the 7-day observation period. In the presence of 0.1–10 nM estradiol (Fig. 1B) the proliferation rates were distinctly increased. When enhancement of MCF-7 cell growth by estradiol was challenged with the simultaneous application of 10 nM MPA (Fig. 1B), the estrogenic stimulation of the proliferation was suppressed. Higher doses than 10 nM estradiol produced minor stimulatory effects in cultures kept in the presence of MPA.

Dose dependence of the inhibitory effect of MPA on the stimulation of cell growth by estrogen or androgen was established in separate series of experiments with simultaneous application of MPA and the steroids (Fig. 2). The growth-rates of MCF-7 cell cultures in 7-day incubation periods were enhanced by more than 100% in the presence of 10 nM estradiol (Fig. 2A) or 1 μM dihydrotestosterone (Fig. 2B). The simultaneous administration of increasing doses of MPA in the range between 1 nM and 1 μM reduced the estrogenic or androgenic stimulation of the proliferation rates nearly to the levels of the untreated control cultures.

Table 1. Medroxyprogesterone-acetate(MPA)-induced suppression of estrogenic or androgenic stimulation of the proliferation in mammary carcinoma cell lines with different growth potential. Alteration of cell number in relation to untreated control cultures (100%) after incubation for 7 days with the indicated hormone concentrations[a]

Cell line	Doubling time[b] (h)	Cell number (% of control)				
		10 nM E_2	10 nM E_2 + 1 μM MPA	1 μM DHT	1 μM DHT + 1 μM MPA	1 μM MPA
MCF-7	42	212	105	229	118	98
EFM-19	110	179	112	169	106	101
MFM-21	41	105	ND	102	ND	95

[a] Representative data of one of at least three experiments. E_2, 17β-estradiol; DHT, dihydrotestosterone; ND, not done
[b] Determined separately from the kinetics of cell growth under standard cultivation conditions

In order to exclude the influence of the inherent potential for different proliferation rates, the reduction of the stimulatory effect of estrogen or androgen by MPA was investigated in slow-growing EFM-19 cell cultures in comparison with the relatively fast-growing MCF-7 cells (Table 1). Under the same experimental conditions, the stimulatory effect of 10 nM estradiol or 1 μM dihydrotestosterone was greatly reduced by the presence of 1 μM MPA in cell cultures of both steroid-sensitive lines, irrespective of their proliferative potential. For comparison, relatively fast-growing MFM-21 human mammary carcinoma cells were resistant to estrogen and to androgen, and their proliferation was not affected by MPA. The results indicate that in these experiments it is only the enhancement of cell growth by estrogen or androgen that is sensitive to interference by MPA.

Down-regulation of the estrogen receptor by MPA

The suppression of growth-stimulatory effects of estrogen and androgen suggests interference of MPA with binding of the steroid hormones to their appropriate receptors. In MCF-7 cultures, direct interaction of MPA with the specific binding of 17β-[^{3}H]estradiol was excluded by experiments in which MPA concentrations up to 1 μM were not competing (data not shown); this is in agreement with earlier observations on the lack of interference of progestins with estrogen binding in MCF-7 cells (Horwitz et al. 1975). However, considerable reduction of estrogen binding, determined by Scatchard analyses, became obvious, when MCF-7 cells were preincubated for 7 days in the presence of 1 μM MPA (Fig. 3). After treatment with high-dose MPA in the absence of estrogenic stimulation, the number of binding sites per cell was reduced by 38% in comparison with untreated cultures. Thus down-regulation of the estrogen receptor was observed in the absence of direct interaction of 1 μM MPA with estrogen binding. The result suggests the existence of a regulatory mechanism reducing estrogen binding by one-third in the presence of 1 μM MPA without any effect on the proliferation of MCF-7 cells (cf. Fig. 1).

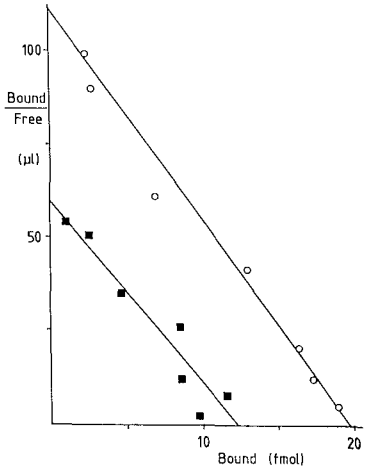

Fig. 3. Scatchard analyses of 17β-[^{3}H]estradiol binding to intact MCF-7 cells demonstrating down-regulation of receptor binding after cultivation for 7 days in the presence of MPA. Specific binding of the radioligand was evaluated in the concentration range of 25 pM to 2.5 nM. In untreated cultures with a specific binding of K_d = 0.18 nM (○), 21700 binding sites/cell were calculated. After preincubation of the cultures with 1 μM MPA (■), the K_d value was unaltered, but the estrogen receptor content was reduced by 38%

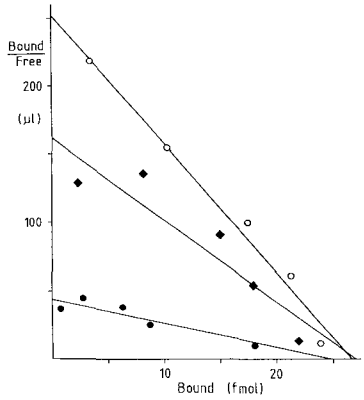

Fig. 4. Competition of MPA with androgen binding in MCF-7 cell cultures. The binding of [^{3}H]R1881 was determined by Scatchard analyses in the concentration range of 25 pM to 2.5 nM resulting in specific binding with K_d = 0.13 nM in untreated cultures (○). 45700 binding sites/cell were calculated. The displacement of [^{3}H]R1881 by 1 nM (◆) or 10 nM (●) MPA yielded apparently reduced affinities, with K_d = 0.17 nM or 0.58 nM, respectively

Competition of MPA with androgen receptor binding

Similar to the experiments in which direct interaction at the estrogen receptor was excluded, the interference of MPA with androgen receptor binding was investigated (Fig. 4). Relatively low doses, such as 1 nM or 10 nM MPA, were able to compete with the artificial androgen [^{3}H]R1881 at the receptor. In the Scatchard analyses, reduced affinities, as indicated by increasing K_d values, were determined. The maximal binding in the short-term assay was nearly constant, underlining the competitive character of the interaction. The affinity of MPA was derived indirectly from these data (K_d = 2.1 nM).

Down-regulation of the androgen receptor by progestins

Reduction of the androgen receptor content was apparent when Scatchard analyses were performed after preincubating MCF-7 cells for 7 days with various concentrations of MPA but under otherwise steroid-free conditions (Fig. 5A). The maximal binding of [^{3}H]R1881, determined from the Scatchard plots, was reduced in a dose-dependent manner. Preincubation of the cell cultures with 1 μM MPA reduced the androgen-binding sites by 64% in comparison with control cultures. The Scatchard plots revealed slightly diminished affinities of the androgen receptor, especially at the 1 μM concentration of MPA during the preincubation period. The slight reduction of binding affinity may be explained by small

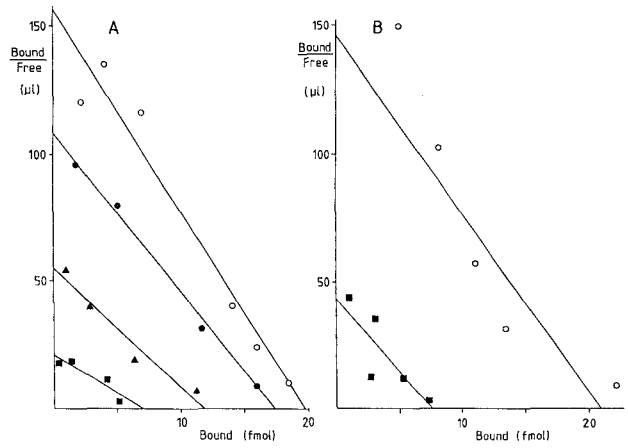

Fig. 5A,B. Down-regulation of the androgen receptor by progestins in MCF-7 cells. **A** After preincubation of the cultures for 7 days with MPA, the maximal binding of the androgen receptor was reduced by 12% at 10 nM (●), by 40% at 100 nM (◆) and by 64% at 1 µM MPA (■). **B** Pretreatment of MCF-7 cell cultures for 7 days with 1 µM synthetic progestin R5020 resulted in reduction of the androgen receptor content to 35% (■) in comparison with the untreated control cultures (○). The K_d values were 0.16 nM in the pretreated and 0.14 nM in the control cultures

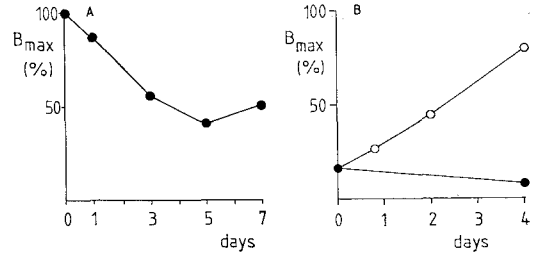

Fig. 6. Time course of the down-regulation (**A**) of the androgen receptor during incubation of MCF-7 cells in the presence of 1 µM MPA (●) and recovery of androgen binding after withdrawal of MPA (**B**). Consecutive up-regulation after a 7-day pretreatment with MPA was determined in a separate series of incubations without MPA (○). Androgen binding was monitored by Scatchard analyses using quadruplicate cell cultures. The results are expressed as percentages of the androgen binding in corresponding, untreated control cultures

amounts of MPA that were not removed completely by the washing procedure preceding the administration of the radioactive ligand in the assay. The reduced affinity after preincubation with 1 µM MPA was used to calculate the concentration of 3 nM MPA (corresponding to 0.3% of the initial concentration) that remained after washing the cultures three times. This is in fair agreement with data obtained by radioimmunoassay in MCF-7 cell cultures incubated separately for 7 days in the presence of 1 µM MPA, which retained 0.22% of the initial MPA concentration after the same washing procedure (Sturm et al. 1988). Progestagenic down-regulation of the androgen receptor was confirmed by application of the synthetic compound R5020 (Fig. 5B), which is known to lack binding to androgen receptors. After preincubation of MCF-7 cell cultures for 7 days with 1 µM R5020, the androgen receptor binding was reduced to 35% of the control. This was nearly the same value as obtained after pretreatment of MCF-7 cells for 7 days with 1 µM MPA (cf. Fig. 5A). In experiments performed with EFM-19 cells (Table 2), down-regulation of the androgen receptor content was slightly less pronounced after preincubation for 7 days with 100 nM MPA. Pretreatment of EFM-19 cell cultures with 1 µM MPA provoked a decrease of the estrogen and androgen receptor content by approximately 60%.

The kinetics of down-regulation of the androgen receptor in MCF-7 cells by 1 µM MPA was followed by up-regulation after release from MPA suppression (Fig. 6). One day after exposure of the cultures to MPA, the reduction of androgen binding became measurable and reached basal values on days 3–7 of the MPA treatment (Fig. 6A). Increase of the androgen receptor binding (Fig. 6B) was observed in cultures that had been preincubated for down-regulation during 7 days in the presence of 1 µM MPA. After withdrawal of MPA from the culture medium, the androgen binding capacity increased gradually and reached nearly the control level 4 days later. Cultures kept in the presence of 1 µM MPA maintained the suppressed state of the androgen receptor. The data of these experiments provide evidence for negative regulatory effects of high doses of progestins on the androgen receptor content under estrogen- or androgen-reduced conditions that are not effective on the cell proliferation rates.

Discussion

In this investigation, the cell growth of the estrogen-sensitive lines MCF-7 and EFM-19 was not affected by doses of 1 nM–1 µM MPA in the absence of estrogen or androgen. In contrast, inhibition of the proliferation of

Table 2. Comparison of the estrogen and androgen receptor content down-regulated in MCF-7 and EFM-19 cell cultures after preincubation for 7 days with MPA

Cell line	Estrogen receptor[a]		Androgen receptor[a]		
	Control	1 µM MPA	Control	100 nM MPA	1 µM MPA
MCF-7	21.700 (100%)	13.450 (62%)	45.700 (100%)	27.400 (60%)	16.500 (36%)
EFM-19	17.800 (100%)	8.000 (45%)	32.900 (100%)	29.200 (89%)	13.900 (42%)

[a] The results obtained from Scatchard analyses are expressed as number of binding sites per cell. Representative data of one of at least three experiments

MCF-7 cells by MPA was reported to occur under comparable estrogen-free, phenol-red-containing, experimental conditions (Braunsberg et al. 1987). This discrepancy may be due to different properties of the MCF-7 substrains explored. Clonal variations of MCF-7 cells were reported to show estrogen-independent growth (Nawata et al. 1981) and resistance to the antiestrogen tamoxifen (Nawata et al. 1981; Reddel et al. 1985). In the present study stimulatory effects of estrogen and androgen on MCF-7 and EFM-19 cell proliferation were suppressed in the presence of MPA. This is in agreement with results (Vignon et al. 1983) reporting major inhibitory effects on the growth of T47D mammary carcinoma cells by the artifical progestin R5020 only in the presence of estrogen. Growth inhibition by progestins may occur without involvement of the estrogen receptor, as was documented recently for MCF-7 cells (Sutherland et al. 1988).

In our study, interference of MPA with estrogen receptor binding was not observed in MCF-7 or EFM-19 cell cultures; this corresponds with the report that progesterone or the synthetic progestin R5020 did not compete with estrogen binding in MCF-7 cultures (Horwitz et al. 1975). Thus interaction of MPA with estrogenic stimulation of cell growth is not based on competition at the estrogen receptor, but may be associated with down-regulation of the estrogen receptor. Down-regulation of the estrogen receptor was found in endometrial carcinoma after MPA treatment (Bonte 1983), it can reach 25% of the controls in MCF-7 cells after a 5-day incubation period with MPA, correlated with a dramatic decrease of [^{3}H]thymidine incorporation (DiMarco 1980). Estrogen receptor mRNA was reduced after incubation with 10 nM R5020 in T47D cells (Read et al. 1989). The inhibition of estrogenic stimulation of cell growth by 1 μM MPA was nearly complete in our experiments, although down-regulation of the estrogen receptor was only to 62% of the controls after incubation with MPA for 7 days. The discrepancy suggests that progestagenic interference with estrogenic stimulation might depend on a threshold value or on additional mechanisms.

In MCF-7 cell cultures, androgens may exert estrogenic effects such as stimulation of the proliferation and induction of the progesterone receptor (Lippman et al. 1976a,b; Hackenberg et al. 1988; Zava and McGuire 1978). In both cell lines MPA prevents androgenic stimulation of the proliferation at comparable concentrations, as shown in this study. Direct interference of MPA with androgen receptor binding was observed in MCF-7 and EFM-19 cell cultures at concentrations of 1 nM and 10 nM MPA. The incubation with higher doses of MPA reduced the number of androgen-binding sites per cell. This effect was dose-dependent, and a reduction by 64% was found after a 7 day-incubation with 1 μM MPA. Down-regulation is independent of binding to the androgen receptor, as shown by equivalent results after incubation with R5020. This is a further example of down-regulation of a steroid-binding protein by another steroid, such as the androgen receptor by estrogen (Stover et al. 1987) and the estrogen receptor mRNA by progestins (Read et al. 1989). Down-regulation of the androgen receptor and competitive replacement at the receptor level may contribute a two-way interference of MPA with the androgenic action in mammary carcinoma cells. Experiments performed to measure down-regulation of the androgen receptor by MPA in the presence of dihydrotestosterone did not yield significant results as dihydrotestosterone interfered with the ligand-binding assay and evidence for autoregulative reduction of the androgen receptor was deduced from diminished quantities of androgen receptor mRNA in male reproductive tissue after 24 h treatment of rats with testosterone propionate (Tan et al. 1988).

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