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Nomegestrol Acetate, a Clinically Useful 19-Norprogesterone Derivative which Lacks Estrogenic Activity

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The estrogenic activity of various 19-norprogestin derivatives has been identified by several laboratories. We have previously hypothesized that the estrogenic activity of these compounds stems from the absence of a methyl group at the 19 position, as various progestins that have a methyl group at this position are not estrogens. To test this hypothesis more directly, we now compare the progestin megestrol acetate against its 19-nor analogue nomegestrol acetate. We also compare these compounds to known estrogens (estradiol, norgestrel, RU486) as well as compounds known to be devoid of estrogenic activity at concentrations as high as 10^{-6} M (medroxyprogesterone acetate, R5020, ICI 182780). In growth assays using the MCF-7 and T47D:A18 human breast cancer cell lines, we find that only estradiol, norgestrel and RU486 stimulate proliferation, and this effect can be blocked by the pure antiestrogen ICI 182780. Furthermore, in transient transfection studies using a luciferase reporter construct containing three tandem copies of the Xenopus vitellogenin A_2 estrogen response element, estradiol, norgestrel and RU486 can stimulate transcription, while none of the other compounds act as estrogens. Transcriptional stimulation by the estrogenic compounds can be blocked by ICI 182780. Our results demonstrate that the lack of a 19-methyl is not the major determinant for estrogenic activity in 19-norprogestins. We suggest that the 17-hydroxyl group more accurately defines estrogenic action.

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INTRODUCTION

The estrogenic activity of 19-norprogestins has been identified by several investigators [1–6]. Our laboratory has compared the ability of various progestins to stimulate the proliferation of the human breast cancer cell line MCF-7 [1, 2, 4, 5] and have found that those progestins lacking a methyl group at the 19 position could stimulate cell growth and estrogen responsive gene transcription. This stimulation could be inhibited by concomitant antiestrogen treatment. Progestins which possessed methyl groups in the 19 position had no stimulatory effect on this cell line. Based upon these findings, we then hypothesized that the 19 position had substantial impact upon the estrogenicity of these progestins [2].

To test the hypothesis that the 19 position is important for the estrogenic action of progestins, we now test the compounds megestrol acetate and nomegestrol acetate which differ only in that the latter lacks a methyl group in the 19 position (Fig. 1). Nomegestrol acetate is a clinically useful progestin [7, 8] and an effective contraceptive agent when used as an implant [9]. A comparable 19-norprogestin used in implanted contraceptive devices (Norplant®) is norgestrel, which we have previously shown to possess estrogenic activity [4]. Botella and colleagues have demonstrated [10] that nomegestrol acetate does not bind to the estrogen receptor and therefore this compound is not likely to possess estrogenic activity. However, estrogen receptor binding assays are somewhat insensitive as progestins proven to possess estrogenic action have been missed using this technique [4, 11]. The growth and transient transfection assays we use can detect estradiol activity at concentrations as low as 10^{-12} M. We therefore used these assay systems to test nomegestrol acetate. We also

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studied known estrogens including estradiol, norgestrel and the antiprogestin mifepristone (RU486) [3–5] as well as compounds known to lack estrogenic activity at concentrations as high as 10^{-6} M including medroxyprogesterone acetate (MPA), promegestone (R5020) and the antiestrogen ICI 182780 (Fig. 1). By comparing these compounds in growth assays, we hope to better define the structural components necessary for the estrogenicity of particular progestins.

We performed the assays described in this paper using the human breast cancer cell lines MCF-7 [12] and T47D:A18 [13]. In previous experiments, we used the MCF-7 cell line as a model system [1, 2, 4, 5]. The T47D:A18 cell line is included not only to determine if our results can be generalized, but also because the T47D:A18 cells dramatically overexpress progesterone receptor [14], and progestins have a small but quantifiable impact on the growth rate of these cells [15, 16].

We also compared the activity of these compounds on transcriptional activation in two human breast cancer cell lines MCF-7 and T47D:A18. These two cell lines were treated with various concentrations of the compounds alone, or in combination with antiestrogen or antiprogestin. Transcriptional activation was measured using a luciferase reporter plasmid containing three tandem copies of the Xenopus vitellogenin A_2 estrogen response element [17]. Our results demonstrate that nomegestrol acetate is devoid of estrogenic activity despite the fact that it lacks a methyl group in the 19 position. By comparing the activity of the other compounds and previous data, we suggest that a 17-hydroxyl group is an important structural feature which determines estrogenic activity of progestins that are 19-nortestosterone derivatives.

MATERIALS AND METHODS

Tissue culture

MCF-7 cells [12] were originally obtained from the Michigan Cancer Foundation. Cells were grown in minimal essential medium containing 5% (v/v) calf serum supplemented with 0.29 mg/ml L-glutamine, 100 U/ml penicillin plus 100 μg/ml streptomycin, 6 ng/ml bovine insulin (Sigma Chemical Co., St Louis, MO), 0.35 g $NaHCO_3$/l, and 25 mM HEPES. T47-D:A18 cells [13] are a clonal cell line derived from T47D cells [14] originally obtained from American Type Culture Collection (Rockville, MD). These cells were grown in RPMI 1640 containing 10% fetal calf serum supplemented with 0.29 mg/ml L-glutamine, 100 U/ml penicillin plus 100 μg/ml streptomycin, 6 ng/ml bovine insulin, 0.35 g $NaHCO_3$/l, and 25 mM HEPES. Cells were harvested by an initial wash with calcium- and magnesium-free Hanks' Balanced Salt Solution, followed by trypsinization. Cells were tested for mycoplasma using GEN-PROBE® rapid detection system (GEN-PROBE Inc., San Diego, CA) every 2 months and all cells were free of mycoplasma.

Hormone treatment

Twenty four well plates were seeded with 15,000 MCF-7 cells in 1 ml per well of phenol red free minimal essential medium with 5% calf serum stripped of estrogen, 0.29 mg/ml L-glutamine, 100 U/ml penicillin plus 100 μg/ml streptomycin, and 6 ng/ml bovine insulin. Serum was stripped using dextran-coated charcoal. T47D:A18 cells were seeded at 20,000 cells per well in 1 ml of phenol red free RPMI with 10% stripped fetal calf serum, 0.29 mg/ml L-glutamine, 100 U/ml penicillin plus 100 μg/ml streptomycin, and 6 ng/ml bovine insulin. The cells were deprived of steroid for 3–4 days. Medium was changed every other day. Compounds were then added at the indicated concentrations, and media with compound were changed every other day for a total of 6 days. All compounds were dissolved in 100% ethanol, and added to the media in 1:1000 dilution for a final ethanol concentration no greater than 0.2%. Estradiol, norgestrel, megestrol acetate and MPA were purchased from Sigma Chemical Co. (St Louis, MO); ICI 182780 was a generous gift from ICI pharmaceuticals (Macclesfield, England); RU486 was a generous gift from Roussel (Romainville, France); nomegestrol acetate and promegestone (R5020) were generous gifts from Dr J. Paris (Théramex, Monaco, Monaco). After the sixth day in the presence of compound, the media was removed and the cells were lysed by sonication for 20 s using a Kontes ultrasonic cell disruptor in 1 ml of calcium- and magnesium-free Hanks' Balanced Salt Solution. Total DNA per well was measured fluorometrically by incubating samples with Hoechst dye 33258 (Calbiochem-Behring Corp., La Jolla, CA) according to a method by LaBarca and Paigen [18] and analyzed on an SLM-Aminco Fluoro-colorimeter III (SLM Instruments, Urbana, IL). Each data point represents a mean of triplicate wells.

Transient transfection analysis

Five hundred thousand cells per well were plated in each well of a 6-well plate (4 ml media per well). 1 day later, the cells were transfected with 1 μg luciferase plasmid per well and 0.5 μg pCMV-β (which constitutively produces β-galactosidase [19]) for normalization. Transfections were done using the calcium phosphate coprecipitation method [20]. 6 h later, the media in the cells was drawn off and media with compound was added. 18 h later, the cells were washed with ice cold PBS and scraped in 200 μl extraction buffer (0.1 M $KHPO_4$ pH 7.5, 1% Triton X-100, 100 mg/ml bovine serum albumin, 2.5 mM phenylmethanesulfonyl fluoride, and 1 mM dithiothreitol). 50 μl of cell extract was added to 350 μl reaction buffer (160 mM $MgCl_2$, 75 mM glycylglycine, pH 7.8, 0.5 mg/ml bovine serum

albumin, 19 mg/ml ATP and 15 mM Tris–HCl, pH 7.5) in a plastic cuvette (Analytical Luminescence Laboratory, San Diego, CA). The luciferase reaction was assayed in a Monolight 2010°C (Analytical Luminescence) using 100 μl of a 0.4 mg/ml luciferin solution in 10 mM $NaCO_3$, pH 6.0. Luciferin potassium salt was obtained from Analytical Luminescence. Cell extracts were assayed for β-galactosidase activity as described previously [21]. Briefly, an aliquot of cell extract is mixed with 1300 μl reaction buffer containing 0.1 M $NaPO_4$, 10 mM KCl, 1 mM $MgSO_4$ (pH 7.0), 2.2×10^{-5} ml β-methylumbelliferone (Molecular probes Inc., Eugene, OR). This mixture was incubated at room temperature for 1 h, and then quenched with 750 μl stop buffer (15 mM EDTA, 0.3 M glycine (pH 11.2)). Samples are read on a LS-5 fluorescence spectrophotometer (Perkin Elmer, Foster City, CA) with an excitation at 350 nm and absorption at 450 nm. Readings are correlated to a standard curve of purified β-galactosidase (Boehringer Mannheim Biochemicals, Indianapolis, IN) and the luciferase units were normalized for β-galactosidase activity. The data shown is a representative experiment with two separate readings per data point.

RESULTS

The compounds used for study are shown in Fig. 1. Synthetic progestins can generally be divided into two groups: the 19-nortestosterone derivatives (represented by norgestrel) and the 17-substituted progesterone compounds (represented by medroxyprogesterone acetate (MPA) and megestrol acetate). We have previously shown that many 19-nortestosterone derivatives (norethindrone, norethynodrel, norgestrel, and gestodene) possess estrogen-like properties [1, 2, 4, 5]. These compounds can stimulate proliferation of the human breast cancer cell line MCF-7, and various antiestrogens can block this stimulation. One structural characteristic common to these compounds is the absence of a methyl group at the 19 position. Because MPA lacked estrogenic action and possessed a 19-methyl, we hypothesized that this position was important for estrogenic action. However, to test this hypothesis, we needed to compare two compounds which differ only in the 19 position. Megestrol acetate and nomegestrol acetate, both 17-substituted progesterone derivatives, provided us with the opportunity to perform the appropriate set of experiments.

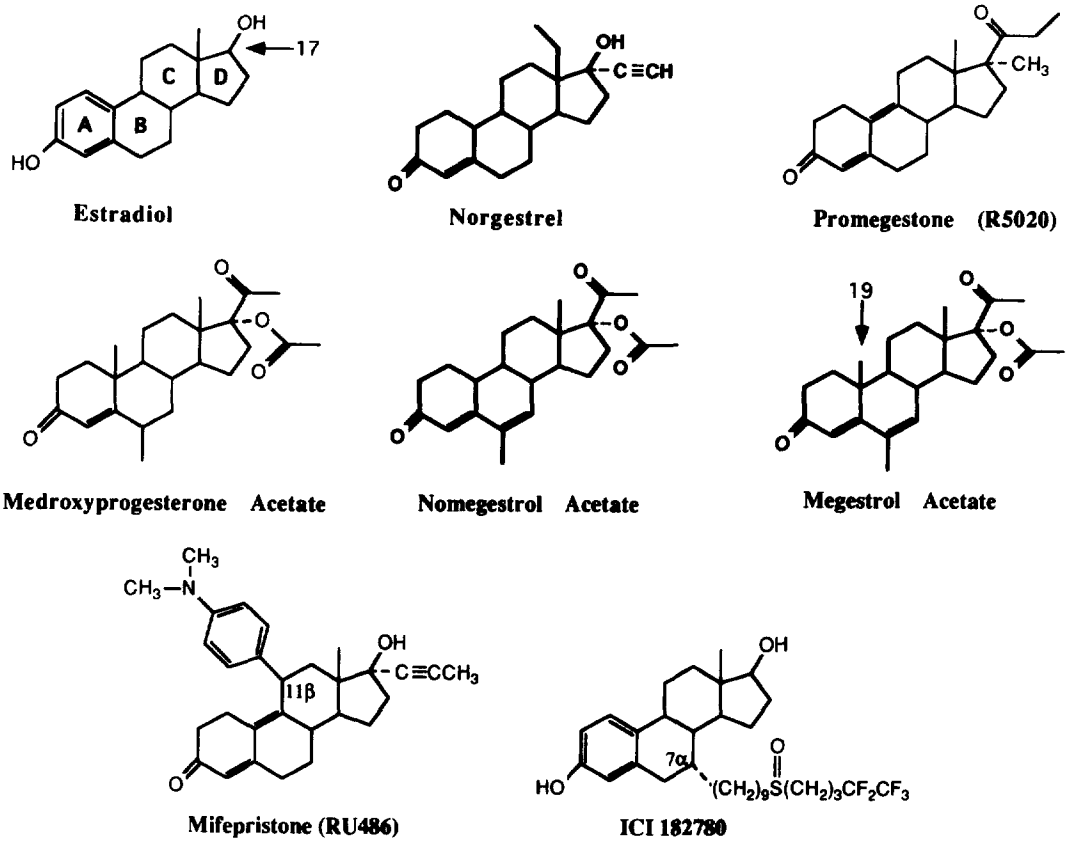

Fig. 1. Molecular structures of compounds used in this study. The ring designations and the 17 position are marked on the molecular structure of estradiol, while the 19 position is identified by an arrow on the structure for megestrol acetate. Note that nomegestrol acetate (center) lacks a 19-methyl group and a 17-hydroxyl. Megestrol differs from nomegestrol acetate only at the 19 position, while the estrogenic progestin norgestrel lacks a 19-methyl but possesses a 17-hydroxyl.

Our results demonstrate that nomegestrol acetate is unable to stimulate proliferation in MCF-7 cells (Fig. 2). Estradiol can stimulate proliferation at concentrations as low as 10^{-12} M, while norgestrel and RU486 provide a growth stimulus at 10^{-7} M. None of the other compounds tested provide a proliferative stimulus [Fig. 2(A)]. The stimulus of estradiol, norgestrel and RU486 can be inhibited by the pure antiestrogen ICI 182780 [Fig. 2(B)]. Combinations of the nonestrogenic progestins and ICI 182780 had no impact on the proliferative rate of MCF-7 cells [Fig. 2(B)], and ICI 182780 has no stimulatory activity alone (data not shown).

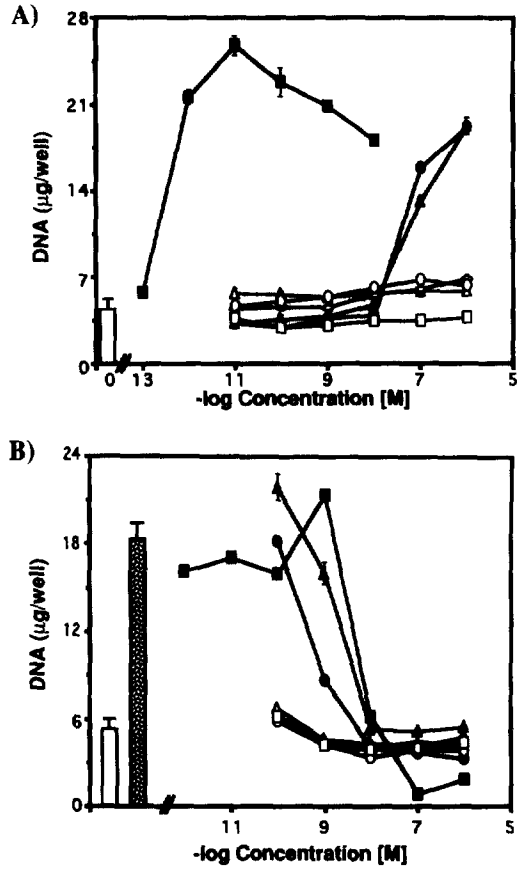

Fig. 2. Proliferative effects of various compounds on MCF-7 human breast cancer cells. (A) Concentration response assays for MCF-7 cells when in the presence of estradiol (■), norgestrel (▲), RU486 (●), nomegestrol acetate (△), megestrol acetate (oa), medroxyprogesterone acetate (○), or promegestone (□). The open bar represents the proliferative activity of MCF-7 cells when in the absence of compound. (B) Competition of proliferative stimulus of various compounds with increasing concentrations of the pure antiestrogen ICI 182780. Competition involves 10^{-6} M of the various progestins or 10^{-10} M estradiol. Estradiol (■), norgestrel (▲), RU486 (●), nomegestrol acetate (△), megestrol acetate (oa), medroxyprogesterone acetate (○), or promegestone (□). The open bar represents the proliferative activity of MCF-7 cells when in the absence of compound, while the speckled bar represents the proliferative activity of MCF-7 cells when in the presence of 10^{-10} M estradiol. ICI 182780 at 10^{-10} M has no inhibitory action on 10^{-6} M treatment with norgestrel or RU486.

While norgestrel can stimulate MCF-7 proliferation, and this stimulation can be inhibited by an antiestrogen, it is difficult to rule out a progesterone receptor mediated mechanism by growth assays. Because the antiprogestin RU486 has estrogenic activity itself, this compound cannot be added to prevent progesterone receptor interaction. Therefore, to guarantee that MCF-7 cell growth stimulation is indeed an estrogen receptor mediated mechanism, we performed transient transfection analyses using a luciferase reporter construct containing three tandem copies of the Xenopus vitellogenin A_2 estrogen response element (Fig. 3). The progesterone receptor is unable to interact with and stimulate transcription from an estrogen response element [22, 23], and as a result stimulation of luciferase by compound addition represents an estrogen receptor mediated mechanism.

Again, nomegestrol acetate is unable to act as an estrogen. Estradiol, norgestrel and RU486 can stimulate luciferase production [Fig. 4(A–C)], while none of the other compounds can activate luciferase transcription. ICI 182780 can block the stimulatory action of estradiol, norgestrel and RU486. These results demonstrate that nomegestrol acetate lacks estrogenic activity, while a different 19-nor compound norgestrel can act as an estrogen.

To ensure that the results seen in previous work [2, 4, 5] and the above experiments were not specific to MCF-7 cells, we performed similar experiments using the T47D:A18 cell line. This estrogen receptor positive human breast cancer cell line dramatically overexpresses progesterone receptor [14], and progestins have a slight impact on the growth rate of these cells. We show in Fig. 5(A) that estradiol and norgestrel stimulate proliferation, while nomegestrol acetate and megestrol acetate have a meager growth stimulatory capability. MPA and R5020 also weakly stimulate cell proliferation (data not shown). When the various compounds are coincubated with ICI 182780, the estrogenic stimulus is inhibited, while the small stimulus produced by all of the progestins remains unblocked [Fig. 5(B)]. RU486 can also stimulate proliferation via the estrogen receptor in T47D:A18 cells at 10^{-7} M (data not shown), and therefore experiments designed to use this antiprogestin to block the stimulus seen by the progestins were confounded. In transient transfection studies using T47D:A18 cells, estradiol and norgestrel could stimulate transcription while nomegestrol acetate and megestrol acetate could not [Fig. 5(C)]. RU486 could also stimulate luciferase activity at 10^{-6} M, while at the same concentration MPA and R5020 could not (data not shown). Stimulation of luciferase transcription could be blocked by ICI 182780. These results demonstrate that nomegestrol acetate is unable to act as an estrogen in MCF-7 and T47D:A18 cells, while a different 19-nor-progestin (norgestrel) acts as an estrogen in both cell lines.

DISCUSSION

We have previously described the estrogenic activity of the progestins norgestrel, norethindrone, norethynodrel, and gestodene [1, 2, 4, 5]. Other groups have also noted the estrogenic activity of other 19-nortestosterone derivatives (gestrinone, Org-OD14, and 7α-methyl-19-nortestosterone) [3, 6]. One structural feature that these compounds have in common is the absence of a methyl group in the 19 position. Based on these findings, we previously hypothesized that the absence of a methyl group in the 19 position could influence the estrogenic activity of progestins. There was, however, contradictory data suggesting that the 19 position may not be directly involved in the estrogenic activity of progestins. Androst-5-ene-3β, 17β-diol is a steroidal compound which possesses a 19-methyl and yet has estrogenic action [24], while R5020 is a 19-norprogestin which lacks estrogenic action except at concentrations higher than 10^{-6} M [2]. Notably, all of the estrogenic progestins possess a hydroxyl in the 17 position, while all of the progestins lacking estrogenic action have bulky ketone and/or acetyl groups at this position. Unfortunately, while the 17 position may be the relevant position for determining estrogenic action, it was impossible to rule out a role for the 19 position until there were compounds developed which differ only at the 19 position.

Nomegestrol acetate provided the opportunity to test this hypothesis. This compound differs only at the 19 position from megestrol acetate, but neither compound possesses a 17-hydroxyl (Fig. 1). Nomegestrol acetate is a clinically useful progestin [7–9] which interacts well with the progesterone receptor, but not with the glucocorticoid [25] or estrogen receptor [26, 27]. Nomegestrol acetate can bind to the androgen receptor and has antiandrogenic activity [28, 29], but is less potent than the androgen testosterone or the antiandrogen cyproterone acetate. When nomegestrol acetate was initially examined in T47D cells, it showed growth inhibitory action [10]. However, these experiments were done in phenol red containing media which has been shown to contain estrogenic contaminants [30], and as a result the direct influence of nomegestrol acetate was unclear. Because our growth and transient transfection assays are virtually devoid of estrogens, they are exquisitely sensitive to any added estrogens. We therefore initiated study to determine the estrogenic activity of nomegestrol acetate, megestrol acetate, MPA, R5020, RU486, and norgestrel in comparison to estradiol.

Our results demonstrate that nomegestrol acetate has no estrogenic action in the MCF-7 and T47D:A18 cell lines. The 19-norprogestins norgestrel and RU486 stimulate breast cancer cell proliferation, and this stimulus can be blocked by the pure antiestrogen ICI 182780. R5020 does not stimulate proliferation, despite its 19-nor designation, further suggesting that the 19 position does not determine estrogenic activity. The estrogenic progestins (norgestrel and RU486) also stimulate estrogen receptor mediated transcription, while the remaining progestins consistently showed no transcriptional activation regardless of the cell line used. Other 19-norprogestins with 17-hydroxyl groups

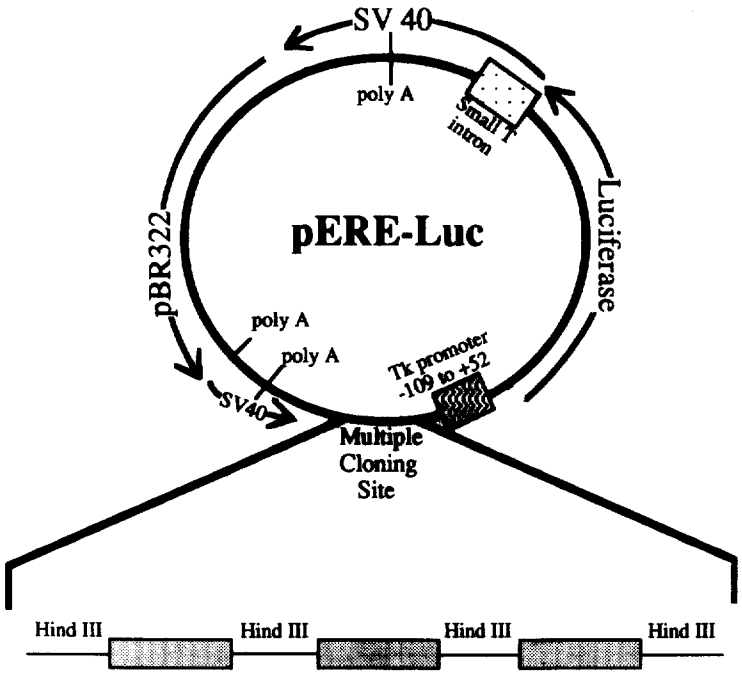

Fig. 3. Plasmid map of a luciferase reporter construct containing three tandem copies of the Xenopus vitellogenin A_2 estrogen response element (GGTCA CAG TGACC) in the promotor region. The stippled bars represent each estrogen response element which are separated by Hind III sites.

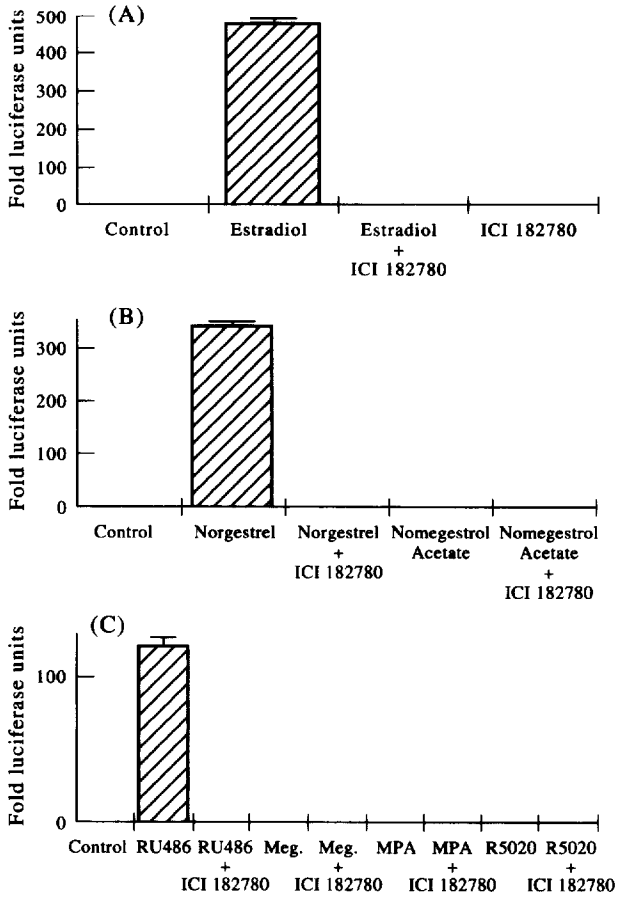

Fig. 4. Transient transfection studies of MCF-7 cells when treated with various compounds. Estradiol was used at 10^{-10} M, while all other compounds were used at 10^{-6} M. All data is represented as fold luciferase production in the absence of compound. (A) Luciferase production by estradiol, ICI 182780, and a competition of estradiol with ICI 182780. (B) Luciferase production by norgestrel and nomegestrol acetate, and competition with ICI 182780. (C) Luciferase production by RU486, megestrol acetate (meg.), medroxyprogesterone acetate (MPA), promegestone (R5020), and competition with ICI 182780.

(norethindrone and gestodene) also stimulate transcription in transient transfection analysis (data not shown). Clearly, the presence or absence of the 19-methyl does not necessarily determine estrogenic action of progestins.

Based on the above results, we suggest that compounds with 17-hydroxyl groups can interact with the estrogen receptor, while ketone side chains and/or acetyl groups in this region of the molecule prohibit estrogen receptor binding and activation. We have previously shown that norethynodrel and norethindrone have greater estrogen-like action compared to norgestrel and gestodene [2, 4]. The difference between the former and latter groups of compounds is a methyl versus an ethyl group in the 18 position. Perhaps the bulkiness of the ethyl group in proximity to the 17 position also inhibits interaction with and activation of the estrogen receptor.

The ideal compound to test the importance of the 17-hydroxyl versus the 19-methyl positions on estrogen-like action would be testosterone, as it is 17-hydroxylated but contains a 19-methyl group. Unfortunately, this compound is aromatized to estradiol intracellularly. As a result, determining the importance of the 17 and 19 positions in the estrogenic activity of certain progestins requires synthesis and characterization of novel steroids.

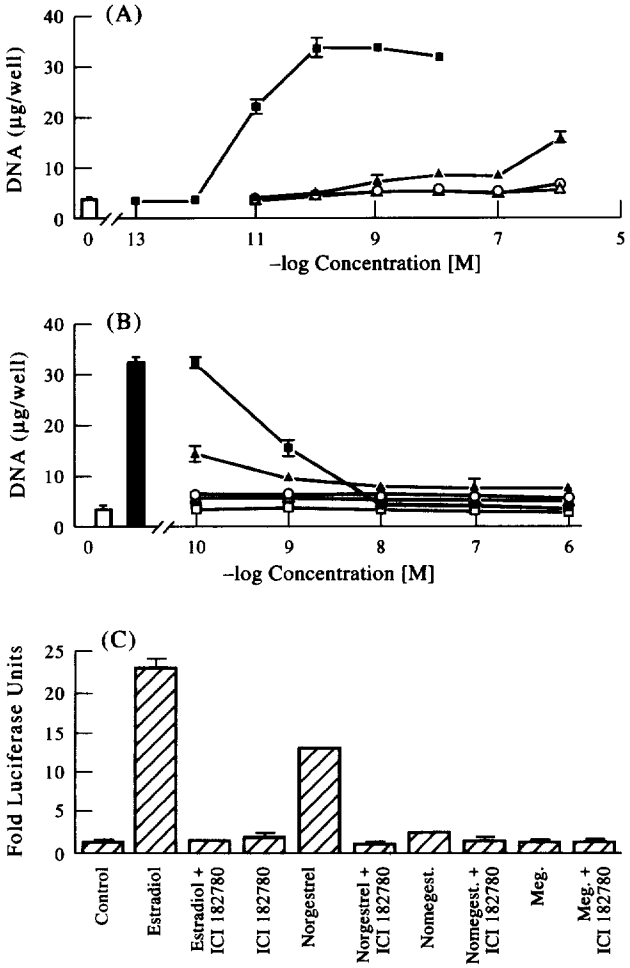

Fig. 5. Growth and transient transfection assays of T47D:A18 cells. (A) Concentration response assays for T47D:A18 cells when in the presence of estradiol (■), norgestrel (▲), nomegestrol acetate (△), or megestrol acetate (○). The open bar represents the proliferative activity of T47D:A18 cells when in the absence of compound. (B) Competition of proliferative stimulus of various compounds with increasing concentrations of the pure antiestrogen ICI 182780. Competition involves 10^{-6} M of the various progestins or 10^{-10} M estradiol. Estradiol (■), norgestrel (▲), nomegestrol acetate (△), or megestrol acetate (○). The open squares represent treatment with ICI 182780. The open bar represents the proliferative activity of T47D:A18 cells when in the absence of compound, while the closed bar represents the proliferative activity of T47D:A18 cells when in the presence of 10^{-10} M estradiol. ICI 182780 at 10^{-10} M has no inhibitory action on 10^{-6} M norgestrel. (C) Transient transfection assays using either 10^{-10} M estradiol, or 10^{-6} M of all other compounds. Each bar represents fold of luciferase production when the cells are in the absence of compound. Nomegest., nomegestrol acetate; meg., megestrol acetate.

The importance of A- and D-rings of steroids for steroid receptor interaction has been described previously [31–36]. Using receptor binding studies to characterize progestin/progesterone receptor interaction, Botella and colleagues [27] demonstrate that removal of the 19-methyl (on the A ring) from testosterone dramatically increases progesterone receptor binding, while having no impact on estrogen receptor interaction. Furthermore, they demonstrate the addition of an acetyl group in the 17α position (on the D ring) to a 17-hydroxyl (nomegestrol acetate vs nomegestrol) also increases progesterone receptor affinity. The 17 and 19 positions are therefore important sites that determine progesterone receptor interaction. Our results suggest that the 17 position may be more relevant than the 19 position for estrogen receptor interaction and activation.

In summary, we demonstrate that the 19-norprogestin nomegestrol acetate (derived from progesterone) does not possess estrogenic activity seen in other 19-norprogestins (which were derived from testosterone). We hypothesize that estrogenic activity is determined by the 17-hydroxyl group associated with estrogenic progestins, rather than the absence of the 19-methyl group. Perhaps the next generation of progestins can benefit from these findings in that a range of estrogenic and nonestrogenic progestins can be tested for clinical activity.

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REFERENCES

1. Jeng M-H. and Jordan V. C.: Growth stimulation and differential regulation of transforming growth factor beta 1 (TGF beta 1), TGF beta 2, and TGF beta 3 messenger RNA levels by norethindrone in MCF-7 human breast cancer cells. *Molec. Endocr.* 5 (1991) 1120–1128.
2. Jeng M-H., Parker C. J. and Jordan V. C.: Estrogenic potential of progestins in oral contraceptives to stimulate human breast cancer cell proliferation. *Cancer Res.* 52 (1992) 6539–6546.
3. Markiewicz L., Hochberg R. B. and Gurpide E.: Intrinsic estrogenicity of some progestagenic drugs. *J. Steroid Biochem. Molec. Biol.* 41 (1992) 53–58.
4. Catherino W. H., Jeng M-H. and Jordan V. C.: Norgestrel and gestodene stimulate breast cancer cell growth through an oestrogen receptor mediated mechanism. *Br. J. Cancer* 67 (1993) 945–952.
5. Jeng M-H., Langan-Fahey S. M. and Jordan V. C.: Estrogenic actions of RU486 in hormone-responsive MCF-7 human breast cancer cells. *Endocrinology* 132 (1993) 2622–2630.
6. Markiewicz L. and Gurpide E.: Estrogenic and progestagenic activities coexisting in steroidal drugs: quantitative evaluation by *in vitro* bioassays with human cells. *J. Steroid Biochem. Molec. Biol.* 48 (1994) 89–94.
7. Fraser D. I., Padwick M. L., Whitehead M. I., White J., Ryder T. A. and Pryse D. J.: The effects of the addition of nomegestrol acetate to post-menopausal oestrogen therapy. *Maruritas* 11 (1989) 21–34.
8. Basdevant A., Pelissier C., Conard J., Degrelle H., Guyene T. T. and Thomas J. L.: Effects of nomegestrol acetate (5 mg/d) on hormonal, metabolic and hemostatic parameters in premenopausal women. *Contraception* 44 (1991) 599–605.
9. Coutinho E. M.: One year contraception with a single subdermal implant containing nomegestrol acetate. *Contraception* 47 (1993) 97–105.
10. Botella J., Duranti E., Duc I., Cognet A. M., Delansorne R. and Paris J.: Inhibition by nomegestrol acetate and other synthetic progestins on proliferation and progesterone receptor content of T47-D human breast cancer cells. *J. Steroid Biochem. Molec. Biol.* 50 (1994) 41–47.
11. Fuhrmann U., Slater E. P., Fritzemeier K-H.: Characterization of the novel progestin gestodene by receptor binding studies and transactivation assays. *Contraception* 51 (1995) 45–52.
12. Soule H. D., Vazquez J., Long A., Albert S. and Brennan M.: A human cell line from a pleural effusion derived from a breast carcinoma. *J. Natn. Cancer Inst.* 51 (1973) 1409–1416.
13. Murphy C. S., Pink J. J. and Jordan V. C.: Characterization of a receptor-negative, hormone-nonesponsive clone derived from T47D human breast cancer cell line kept under estrogen-free conditions. *Cancer Res.* 50 (1990) 7285–7292.
14. Keydar I., Chen L, Karby S., Weiss F. R., Delarea J., Radu M., Chaitcik S., and Brenner H. J.: Establishment and characterization of a cell line of human breast carcinoma origin. *Eur. J. Cancer* 15 (1979) 659–670.
15. Horwitz K. B. and Freidenberg G. R.: Growth inhibition and increase of insulin receptors in antiestrogen resistant T47Dco human breast cancer cells by progestins: implications for endocrine therapies. *Cancer Res.* 45 (1985) 167–173.
16. Musgrove E. A., Lee C. S. and Sutherland R. L.: Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor alpha, epidermal growth factor receptor, c-*fos*, and c-*myc* genes. *Molec. Cell. Biol.* 11 (1991) 5032–5043.
17. Klein-Hitpaß L., Schorpp M, Wagner U. and Ryffel G. U.: An estrogen-responsive element derived from the 5′ flanking region of the Xenopus vitellogenin A_2 gene fuctions in transfected human cells. *Cell* 46 (1986) 1053–1061.
18. LaBarca C. and Paigen K.: A simple, rapid, and sensitive DNA assay procedure. *Analyt. Biochem.* 102 (1980) 344–352.
19. MacGregor G. and Caskey C. T.: Construction of plasmids that express *E. coli* beta-galactosidase in mammalian cells. *Nucl. Acids Res* 17 (1989) 2365.
20. Sambrook J. Fritsch E. F. and Maniatis T. (Eds): *Molecular Cloning. A Laboratory Manual*, 2nd Edition. Cold Spring Harbor Laboratory Press, New York (1989).
21. Luyten G. P., Hoogeveen A. T. and Galjaard H.: A fluorescence staining method for the demonstration and measurement of lysosomal enzyme activities in single cells. *J. Histochem. Cytochem.* 33 (1985) 965–968.
22. Klein-Hitpass L., Tsai S. Y., Greene G. L., Clark J. H. Tsai M. J. and O'Malley B. W.: Specific binding of estrogen receptor to the estrogen response element. *Molec. Cell. Biol.* 9 (1989) 43–49.
23. Bradshaw M. S., Tsai S. Y., Leng X., Dobson A. D. W., Conneely O. M., O'Malley B. W. and Tsai M-J.: Studies on the mechanism of functional cooperativity between progesterone and estrogen receptors. *J. Biol. Chem.* 266 (1991) 16,684–16,690.
24. Poulin R. and Labrie F.: Stimulation of cell proliferation and estrogenic response by adrenal C_{19}-Δ^5-steroids in ZR-75-1 human breast cancer cell line. *Cancer Res.* 46 (1986) 4933–4937.
25. Botella J., Porthe-Nibelle J., Paris J. Lahlou B.: Interaction of new 19-nor progesterone derivatives with progestagen, mineralocorticoid and glucocorticoid cytosolic receptors. *J. Pharmac.* 17 (1986) 699–706.
26. Botella J., Duc I., Delansorne R., Paris J. and Lahlou B.: Regulation of rat uterine steroid receptors by nomegestrol acetate, a new 19-nor-progesterone derivative. *J. Pharmac. Exp. Ther.* 248 (1989) 758–761.
27. Botella J., Duc I, Delansorne R., Paris J. and Lahlou B.: Structure–activity and structure–affinity relationships of 19-norprogesterone derivatives in rat uterus. *J. Endocr. Invest.* 13 (1990) 905–910.

28. Botella J., Paris J. and Lahlou B.: The cellular mechanism of the antiandrogenic action of nomegestrol acetate, a new 19-norprogestagen, on the rat prostate. *Acta Endocr.* **115** (1987) 544–550.
29. Duc I., Botella J., Bonnet P., Fraboul F., Delansorne R. and Paris J.: Antiandrogenic properties of nomegestrol acetate. *Arzneim. Forsch./Drug Res.* **45** (1995) 70–74.
30. Berthois Y., Katzenellenbogen J. A. and Katzenellenbogen B. S.: Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 2496–2500.
31. Duax W. L., Cody V. and Hazel J.: Steroid structure and function:I. Conformational transmission in 17alpha-acetoxy progesterone. *Steroids* **30** (1977) 471–480.
32. Duax W. L., Cody V., Griffin J. F., Rohrer D. C. and Weeks C. M.: Molecular conformation and protein binding affinity of progestins. *J. Toxic. Environ. Health* **4** (1978) 205–227.
33. Duax W. L. Cody V., Griffin J., Hazel J. and Weeks C. M.: Steroid structure and function—II. Conformational transmission and receptor binding of medroxyprogesterone acetate. *J. Steroid Biochem.* **9** (1978) 901–907.
34. Schmit J. P. and Rousseau G. G.: Structure–activity relationships for glucocorticoids—III. Structural and conformational study of the rings and side chain of steroids which bind to the glucocorticoid receptor. *J. Steroid Biochem.* **9** (1978) 909–920.
35. Duax W. L. and Strong P. D.: Steroid structure and function. V. A-ring conformation in 17-hydroxy-6 alpha-methylprogesterone. *Steroids* **34** (1979) 501–508.
36. Schmit J. P., Quivy J. I. and Rousseau G. G.: The androgen receptor binding site: a conformational study of steroids in relation with their affinity-I. Interaction with the D-ring. *J. Steroid Biochem.* **13** (1980) 1387–1394.