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Inhibition by Nomegestrol Acetate and Other Synthetic Progestins on Proliferation and Progesterone Receptor Content of T47–D Human Breast Cancer Cells

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Progesterone receptors (PgR) of human breast cancer T47-D cells grown in an estrogenic environment (presence of phenol red, natural estrogens of foetal calf serum and insulin) were found to be present in considerable amounts (1–3 pmol/mg protein and 20 pmol/mg DNA), and to specifically bind progestins with a high affinity characterized by a K_d around 3 nM for ORG2058, and 4 nM for nomegestrol acetate (NOM; 17α-acetoxy-6-methyl-19-nor-pregna-4,6-diene-3,20-dione), when measured under equilibrium conditions. Both compounds formed an highly stable ligand–receptor complex with a dissociation constant (k_{-1}) around $1 \times 10^{-5}\ s^{-1}$. At high pharmacological concentrations, NOM, ORG2058 and other synthetic progestins including promegestone (R5020), medroxyprogesterone acetate and norethindrone acetate (NOR), induced a dose-dependent inhibition of cell proliferation as measured by [^{3}H]thymidine incorporation. Dexamethasone, which did not bind to PgR, did not reproduce this inhibitory effect. NOM, R5020 and NOR treatments of T47-D cells at concentrations around K_d resulted in an 80% decrease in PgR content. Our data on NOM as compared to other progestins are consistent with their antiproliferative effects on human breast cancer cells grown in estrogenic conditions.

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INTRODUCTION

The concept that estrogens are mitogenic on established breast cancer cell lines is now well established [1]. Conversely, the other ovarian steroid hormone, progesterone (P), and its synthetic derivatives are thought to be protective, based on their antiestrogenic and therefore antiproliferative effects on endometrium [2].

The synthetic progestin medroxyprogesterone acetate (MPA) is commonly employed in the treatment of hormone-responsive breast cancer tumours. Either used alone, sequentially, or in combination with the antiestrogen tamoxifen (TAM), MPA treatment results in an objective response in at least one third of patients [3]. In patients with progesterone receptor (PgR)-positive tumours, the response to progestin therapy can be as high as 70% [3].

Nomegestrol acetate (NOM), a synthetic 19-norprogesterone derivative largely used in non-tumoural pathology was previously found to negatively regulate estradiol-induced PgR levels in uterus from castrated rats [4] as well as from immature rabbits [5]. We have investigated the effects of NOM and other synthetic progestins on PgR levels under conditions of progestin-induced growth inhibition on the human breast cancer cell line T47-D, i.e. in an estrogenic environment. Those cells have previously been shown to be growth inhibited in a concentration- and time-dependent fashion by other progestins such as MPA and antiestrogens such as 4-OH-tamoxifen [6]. The cytosolic PgR in T47-D cells have been demonstrated to be present in extraordinary amounts (2 pmol/mg protein and 10–20 pmol/mg DNA) and to retain normal characteristics : they are functional, can be translocated to, and quantitatively recovered from nuclei after P addition [7, 8]. The levels of those PgR are down-regulated by progestins and this decrease has been shown to result from a PgR-mediated shortening of the PgR protein

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half-life, and from a drop in PgR mRNA concentration [9].

The present study was undertaken to document the effect of NOM, as compared to other well known progestins, on T47-D cells, a highly PgR-positive human breast cancer cell line in culture.

MATERIALS AND METHODS

Materials

Organon 2058 (ORG2058; 16α-ethyl-21-hydroxy-19-norpregn-4-en-3,20-dione), [^{3}H]ORG2058 (50 Ci/mmol) and [^{3}H]thymidine (85 Ci/mmol), were obtained from Amersham (Les Ulis, France). Promegestone (R5020; 17α, 21-dimethyl-19-norpregn-4,9 dien-3,20-dione) and [^{3}H]R5020 (86 Ci/mmol) were purchased from NEN, Du Pont de Nemours (Paris, France). Norethindrone acetate (NOR; 17α-ethynyl-17β-acetoxy-4-estren-3-one), MPA (17α-acetoxy-6α-methyl-pregn-4-en-3,20-dione), P, dexamethasone (DEX; 9α-fluoro-16-methyl-prednisolone) and 17β-estradiol (E_2) were from Sigma (St-Quentin Fallavier, France).

All media, sera and antibiotics for cell culture were obtained from GIBCO (Cergy Pontoise, France). Other reagents were from Sigma. NOM (17α-acetoxy-6-methyl-19-norpregn-4,6-dien-3,20 dione) was synthesized by Theramex (Monaco) and labelled as [^{3}H]NOM (4.4 Ci/mmol) by Amersham (Amersham, England).

Cell culture

T47-D cells obtained from the European Collection of Animal Cell Cultures (ECACC, No. 85102201, Salisbury, England) were plated in Nunc plastic flasks (75 to 175 cm^2). The cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing L-glutamine (4 mM), glucose (4.5 g/l) and phenol red (15 mg/l), and supplemented with penicillin–streptomycin (5000 IU/l), insulin (0.6 μg/ml), transferrin (5 μg/ml) and 5% of decomplemented foetal calf serum (FCSd; heat inactivation for 1 h at 56°C). Cells were maintained in a humidified atmosphere of 7% CO_2 and 93% air at 37°C. When needed, steroids were dissolved in ethanol and added to the culture medium (0.1% v/v) to obtain the final concentrations described in the legend of the figures. Ethanol (0.1% v/v) was also added to control cells. The cells used in these studies were from passages 10 to 40 in our laboratory. Stocks were passaged once a week to maintain continuous exponential growth. Cells were plated in 175 cm^2 flasks for binding studies or in 24- or 48-well microplates for proliferation and PgR content experiments.

Binding assay: cytosol extracts from untreated cells

On the day of the binding assay, the culture medium was removed from the flasks and the cells were washed twice with ice-cold phosphate buffered saline (PBS), harvested by scraping in TEG buffer (10 mM Tris, 20 mM sodium molybdate, 1.5 mM EDTA, 1 mM dithiotreitol, 10% glycerol, pH 7.4 with HCl; 2 ml/175 cm^2 flask) and collected as a suspension. The cell contents of four flasks were pooled and homogenized in an all glass Dounce apparatus. The homogenate was centrifuged at 105,000 *g* for 1 h at 4°C. The supernatant cytosol was diluted in TEG buffer (1.5- to 2-fold) and was immediately used in the PgR binding assay. The cytosolic protein concentrations were determined by the method of Bradford [10] using bovine serum albumin (BSA) as a standard; they routinely ranged from 0.5 to 1 mg/ml.

Saturation analysis

Cytosol samples were incubated with increasing concentrations of [^{3}H]NOM or [^{3}H]ORG2058 as described in the legend of the figures. Parallel sets contained a 200-fold molar excess of P for non-specific binding (NSB) determination. After 3 h of incubation at 4°C, bound and free steroid fractions were separated by incubation with dextran-coated charcoal (DCC) in Tris buffer (10 mM Tris, 1.5 mM EDTA, 2.5% charcoal, 0.25% dextran; pH 7.4 with HCl) for 10 min at 4°C. Incubations with DCC were terminated by centrifugation at 800 *g* for 10 min, and aliquots were submitted to radioactive counting of the receptor-bound fraction. Data were plotted according to the method of Scatchard [11] after NSB substraction.

Determination of the dissociation rate constant, k_{-1}

According to Schrader and O'Malley [12], cytosols were incubated in the presence of 30 nM of ^{3}H-ligand with (NSB) or without (Bo) a 200-fold molar excess of P. After a 24 h association period, aliquots of the labelled cytosols were incubated further with the corresponding non-labelled progestin. At various times thereafter, DCC was applied and specific binding (B) measured. Semilogarithmic plots of B/Bo versus time gave straight lines, the slope of which were k_{-1}. Parallel incubations were made without competitor to check the stability of [^{3}H]-steroid–PgR complexes.

Specificity of progestin binding to T47-D cells PgR

Cytosols were incubated at 4°C with a ^{3}H-ligand (ORG2058, R5020 or NOM) for either 3 or 24 h alone or in the presence of unlabelled steroids (P and related compounds). NSB was measured in parallel in the presence of a 200-fold molar excess of P. Samples were then submitted to DCC separation and radioactive counting.

Cell proliferation and DNA synthesis

The effect of progestins and DEX on [^{3}H]thymidine incorporation by T47-D cells was tested in the presence of phenol red. Cells were plated at a density of 10^4/cm^2 in 24-well microplates. The next day, the medium was changed and test substances were added

in a small volume of culture medium (0.1% ethanol v/v). The incubation medium and the test substances were renewed 3 days later. After an additional 3 day period of incubation, DNA synthesis was measured. [^{3}H]thymidine (0.1 μCi/well) was allowed to incorporate for 2 h at 37°C. At the end of the incubation, plates were layered on ice and wells were rinsed with ice-cold PBS. Cells were precipitated with cold trichloroacetic acid (TCA, 10%) for 10 min at 4°C, then pelleted after elimination of TCA and lysed with NaOH (0.1 N) during 30 min. An aliquot of NaOH solution was submitted to radioactive counting.

PgR level assay

A whole cell PgR assay was performed using a single saturating dose of [^{3}H]ORG2058. T47-D cells (4000/cm^2) were plated in 48-well microplates and treated as described above. Subsequently, the medium was removed, and the cells incubated for 2 h at 37°C in the same fresh medium containing 20 nM of [^{3}H]ORG2058 in the absence (total binding) or presence (NSB) of a 200-fold excess of R5020. At the end of the assay, the cells were washed 3 times with PBS containing BSA (1 mg/ml), and twice with PBS without BSA. Then, the cells were lysed according to the method of West *et al.* [13] using a basic buffer (10 mM EDTA, pH 12.3 with NaOH) for 30 min at 37°C. Next, plates were layered on ice and an iced-buffer (KH_2PO_4, 1 M) added for neutralization. An aliquot was submitted to radioactive counting. Another aliquot was taken for DNA determination according to the method of Labarca and Paigen [14] by fluorescent staining with Hoechst 33258.

Statistical analysis

Data are reported as the mean ± SEM and analysed by one-way analysis of variance and Student's t-test.

RESULTS

NOM binds to T47-D cells PgR with high affinity

Typical concentration–response curves (Fig. 1) for [^{3}H]NOM and [^{3}H]ORG2058 binding to cytosol receptors showed that 20 nM was saturating; this concentration was subsequently used in a single saturating dose, DCC-assay, to determine PgR levels. When data were plotted according to the method of Scatchard [11], a single class of high-affinity binding sites was demonstrated, with a K_d of the same order of magnitude for the two ligands: from 1.7 to 6.6 nM for [^{3}H]NOM and from 1.0 to 4.1 nM for [^{3}H]ORG2058 (Table 1). The number of binding sites (B_{max}) ranged from 1.0 to 2.8 pmol/mg proteins (Table 1). The NSB was < 10% of the total binding.

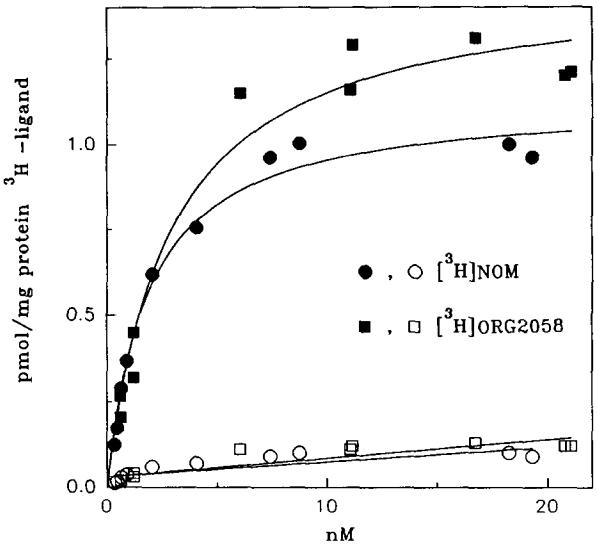

Fig. 1. Binding of [^{3}H]NOM (●) and [^{3}H]ORG2058 (■) to T47-D cells cytosol. Triplicate aliquots of cytosols (0.6 mg/ml of protein) were incubated at 4°C for 3 h with 0.4 to 20.0 nM of [^{3}H]NOM or [^{3}H]ORG2058 for the determination of total binding. Incubations with ^{3}H-ligand in the presence of a 200-fold molar excess of P were made in parallel for the determination of non-specific binding (NSB, open symbols). The specifically bound radioactivity (SB, closed symbols) was plotted after substraction of NSB. Each value is the mean of a triplicate determination, for a pool of 2 representative experiments.

NOM dissociates slowly from the T47-D cells PgR

The results from the kinetic studies are summarized in Table 1. NOM and ORG2058 dissociation rate constants (k_{-1}) from the cytosolic PgR did not differ markedly from each other: 0.68 ± 0.12 vs 1.08 ± 0.12 10^{-5} s^{-1}, respectively. The k_{-1} was also estimated for R5020: 1.04 ± 0.07 10^{-5} s^{-1}.

Table 1. Dissociation constant (K_d), maximal number of binding sites (B_{max}) and dissociation rate constant (k_{-1}) for the interaction between labelled NOM or ORG2058 and PgR in T47-D cells

	[^{3}H]NOM	[^{3}H]ORG2058
K_d (nM)	3.65 ± 0.54 (7)	2.65 ± 0.44 (6)
B_{max} (pmol/mg proteins)	1.61 ± 0.12 (7)	1.02 ± 0.32 (6)
k_{-1} (s^{-1})	0.68 ± 0.12 × 10^{-5} (6)	1.08 ± 0.12 × 10^{-5} (5)

Means ± SEM for (n) determinations. K_d, B_{max} and k_{-1} of the two ligands were not statistically different from each other.

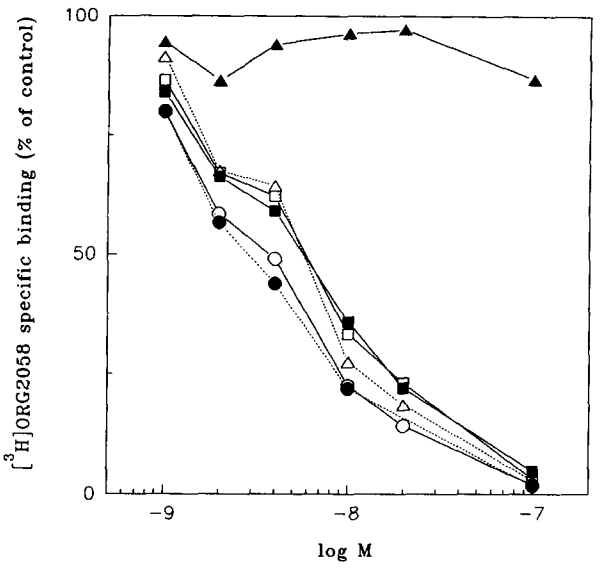

Fig. 2. Specificity of NOM binding to T47-D cell PgR. Cytosol (0.6 mg/ml of protein) from untreated cells was incubated for 3 h at 4°C with 2 nM [^{3}H]ORG2058 alone or together with 10^{-9} to 10^{-7} M of unlabelled steroids [DEX (▲), P (■), R5020 (△), ORG2058 (●), MPA (□), NOM (○)]. [^{3}H]ORG2058 binding was determined by DCC assay as described in Materials and Methods. Non-specific binding was assessed by a 200-fold molar excess of P. Data points: means of triplicate measurements from three to four separate experiments. Standard errors (not shown) ranged from 0.2 to 5.7% of the mean.

Table 3. IC_{50} of NOM, ORG2058 and R5020 binding to PgR in T47-D cells, competition against 4 nM of [^{3}H]NOM, [^{3}H]ORG2058 and [^{3}H]R5020 after 24 h incubation

	NOM	ORG2058	R5020
[^{3}H]NOM[a]	3.71 ± 0.34 (7)	3.76 ± 0.38 (6)	3.42 ± 0.34 (5)
[^{3}H]ORG2058[b]	4.54 ± 0.63 (5)	5.28 ± 0.51 (5)	8.04 ± 0.94 (5)
[^{3}H]R5020[a]	5.15 ± 0.91 (4)	4.10 ± 0.64 (4)	6.67 ± 1.27 (4)

Means ± SEM for (n) determinations. [a]IC_{50} for the 3 unlabelled ligands were not different from each other. [b]IC_{50} for R5020 was different from that of ORG2058 and that of NOM ($P < 0.05$).

unlabelled homologues. Results at 24 h (Table 3) showed that the three ligands were approximately equipotent, in spite of a slightly lower ability of R5020 to displace [^{3}H]ORG2058 ($P < 0.05$) already observed after shorter incubations (3 h).

NOM inhibits the growth of T47-D cells

Non-specific effects on cell proliferation were observed with E_2 and/or NOM at the highest concentration tested: 10^{-5} M. At lower concentrations, E_2 alone did not modify the growth of T47-D cells, whereas NOM alone was able to reduce it (Fig. 3). This inhibitory activity of NOM alone was obtained with concentrations as low as 10^{-9} M, but remained relatively weak with only −50% of control levels at

NOM binding is specific of PgR in T47-D cells

The specificity of binding to the receptor was assessed in 3 h-competition studies by incubating cytosols with 2 nM [^{3}H]ORG2058 together with unlabelled hormones in concentrations covering a 2-log range (Fig. 2). DEX, a synthetic glucocorticoid, failed to compete for binding. The ability to compete with [^{3}H]ORG2058 was shown to follow a decreasing order among the five progestagenic compounds tested: ORG2058 ≥ NOM > P ≥ R5020 ≥ MPA (Table 2). In another set of experiments, long term competition studies were performed using [^{3}H]NOM, [^{3}H]ORG2058 and [^{3}H]R5020 and their respective

Table 2. Relative binding affinities (RBA) of steroids for PgR in T47-D cells, competition against 2 nM of [^{3}H]ORG2058 for 3 h at 4°C

	IC_{50} nM	RBA
ORG2058	2.86 ± 0.28 (3)	115.0 ± 29.8
NOM	3.55 ± 0.38 (11)	100%
P	5.66 ± 0.46 (3)*	57.2 ± 13.2
R5020	5.80 ± 0.76 (4)*	64.0 ± 7.8
MPA	5.95 ± 0.67 (4)**	67.8 ± 20.8

Means ± SEM for (n) determinations. IC_{50} is the approximate concentration of competitor causing 50% inhibition of [^{3}H]ORG2058 binding; *$P < 0.05$; **$P < 0.01$ from NOM.

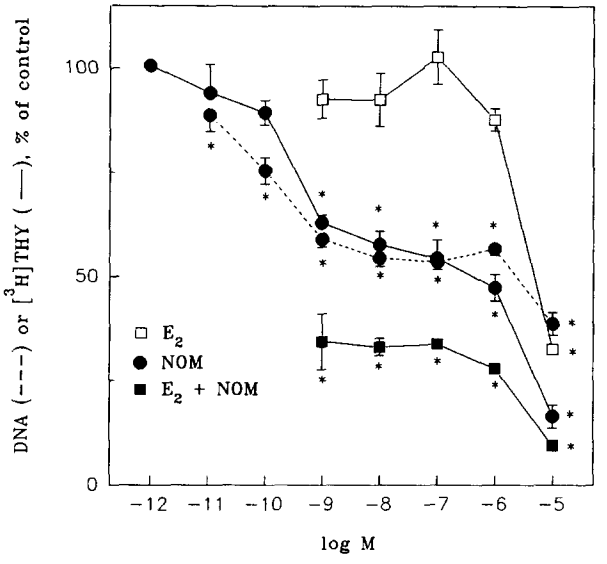

Fig. 3. Effect of NOM and E_2 on the growth of T47-D cells: concentration–response curves. Exponentially growing T47-D cells were plated in 24- or 48-well dishes in DMEM medium containing 5% FCSd in the presence of insulin. After 1 day, cells were treated with NOM alone, E_2 alone or NOM + 1 nM E_2. Media were replaced 3 days later, and after an additional 3 day period, cells were processed for total DNA (---) or [^{3}H]thymidine incorporation (——) assay. Results are expressed in % of control DNA or [^{3}H]thymidine values (without NOM and without E_2). Each point represents the mean ± SEM of 5 determinations, i.e. 5 microplates each made in duplicate ([^{3}H]thymidine) or sextuplicate (DNA) wells. *$P < 0.05$ as compared to control.

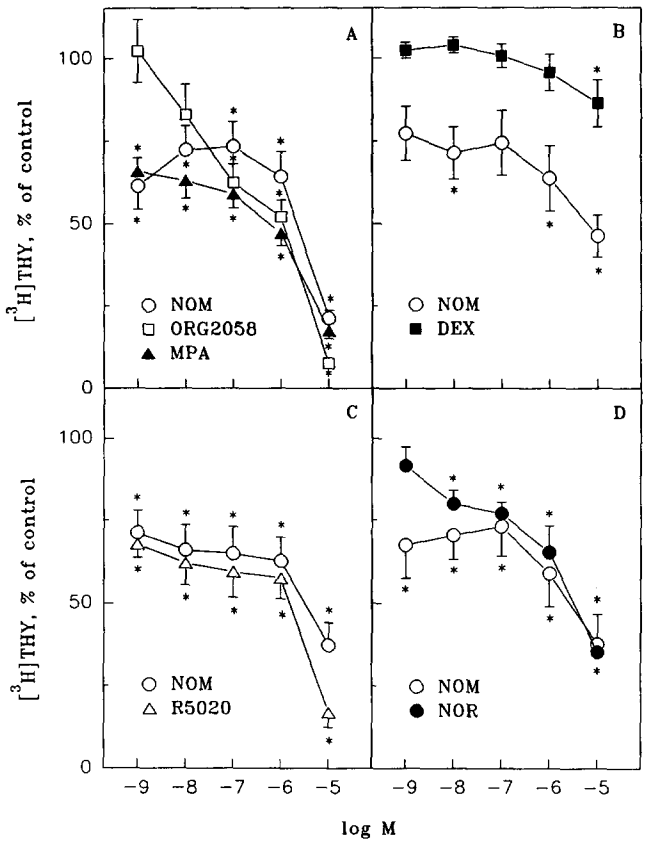

Fig. 4. Comparative effects of steroids on [^{3}H]thymidine incorporation in T47-D cells. Exponentially growing cells were plated in 24-well dishes as described in the legend of Fig. 3. NOM was studied in parallel with ORG2058 and MPA (A), or with DEX (B), R5020 (C), or NOR (D). Every point represents the mean ± SEM of 5 determinations, i.e. 5 microplates each made in duplicate wells. *$P < 0.05$ as compared to control.

10^{-6} M. In combination with 1 nM of E_2 the NOM-induced growth inhibition was potentiated to −70% of control levels.

It should be noted that the two methods used to measure cell proliferation (DNA fluorescent assay and [^{3}H]thymidine incorporation) gave near identical results.

The effect of NOM on T47-D cell growth appears to be specific to PgR

The effect of NOM was then compared to that of DEX which did not bind PgR (Fig. 2) and of other progestins, among which ORG2058 which possesses the same binding characteristics than NOM to PgR (Figs 1 and 2). DEX did not significantly inhibit [^{3}H]thymidine incorporation, while all the four tested progestins reduced the cell growth in a way similar to that of NOM (Fig. 4). In each assay, no significant difference was observed between NOM and ORG2058, R5020, MPA or NOR. In addition, it appeared that the effect of NOM was reproducible independently of the cell passage number:10 to 12 [Fig. 4(A)], 20 to 23 [Fig. 4(B and C)] and 25 to 30 [Fig. 4(D)].

NOM inhibits PgR levels in T47-D cells

NOM, NOR and R5020 sharply inhibited PgR levels in T47-D cells from a value as high as 19.7 ± 0.3 (24) pmol/mg DNA down to levels around 4.4 ± 0.3 (19) pmol/mg DNA with 10^{-8} M of any progestin (Fig. 5). The half maximal inhibition occurred between 10^{-11} and 10^{-10} M, a concentration far lower than the value found for the half maximal inhibition of [^{3}H]thymidine incorporation (between 10^{-7} M and 10^{-6} M). The maximal effect on PgR levels was obtained at 10^{-8} M with a decrease to around 20% of control values, a level which could not be further reduced with increasing progestin concentrations up to 10^{-6} M.

DISCUSSION

The T47-D cells remained stable in terms of responses to NOM for at least 40 weekly passages in our laboratory. When incubated for 3 h with either [^{3}H]NOM or [^{3}H]ORG2058, the cytosolic extracts of T47-D cells revealed an exceptionally large number of a single class of binding sites (B_{max} between 1 and 3 pmol/mg proteins), the affinity of which was high for both progestins and conform to values reported in the literature: 4.2 nM for R5020 or P [7] and 1.0 nM for ORG2058 [3]. RBA studies against [^{3}H]ORG2058 gave

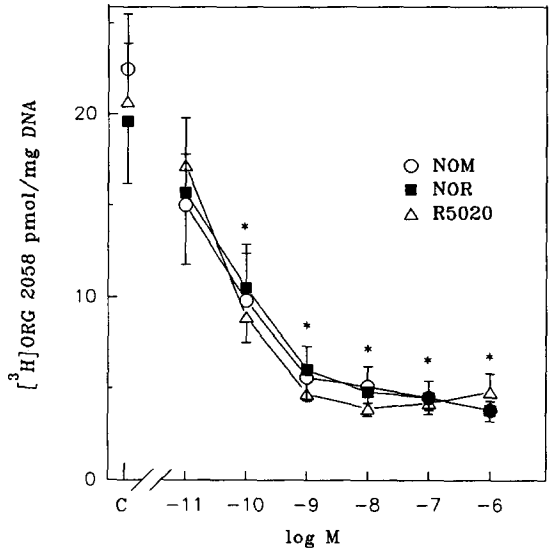

Fig. 5. Effect of progestins on PgR levels in T47-D cells. Exponentially growing cells were plated in 48-well dishes in DMEM medium containing 5% FCSd in the presence of insulin. After 1 day, cells were treated with increasing concentrations of progestins and media were replaced 3 days later. After an additional 3 day period, cells were processed for PgR and DNA assays in the same wells. Each point represents the mean ± SEM of 5 determinations, i.e. 5 microplates each made in triplicate wells. *$P < 0.05$ as compared to control (C).

similar results after short (3 h) and long (24 h) incubations, confirming that K_d and B_{max} measurements were already performed under equilibrium conditions at 3 h [7]. In both cases, NOM and ORG2058 were not different from each other, but displayed a slightly higher affinity than R5020 for T47-D cells PgR.

Futhermore, the three potent ^{3}H-ligands used: NOM, ORG2058 and R5020 dissociated very slowly, and in a similar fashion from this protein–steroid complex. Since DEX, a synthetic glucocorticoid, failed to compete for binding, as reported by others [7], we conclude that, despite the extremely high level of specific binding, the receptors we were measuring were entirely PgR. In summary, NOM binding to PgR in this cell line of human origin was described for the first time and found to be similar to that of ORG2058 in terms of K_d, B_{max}, k_{-1} and RBA, and very close to that of R5020.

The present study has also clearly demonstrated that NOM as other progestins like MPA [15], can inhibit the growth of T47-D human breast cancer cells. However, we did not find the mitogenic effect of E_2 which, according to the cell line under analysis, is able to stimulate the proliferation of established breast cancer cell lines either completely as for ZR-75 or MCF-7 (McGrath) or partially as with T47-D or MCF-7 (Ko) [1]. Rueld *et al.*, [16] studied the effect of E_2 on T47-D cell proliferation in different culture conditions and demonstrated that the dose-dependent mitogenic action of E_2 was not detectable when the culture medium was supplemented with insulin. So, the presence of insulin in our culture media may explain why we did not obtain any proliferation in response to E_2. It is also valuable to remember that the pH indicator, phenol red, which is present in the culture media of our present studies, is estrogenic for human breast cancer lines [17]. In this weak estrogenic environment (phenol red and natural estrogens present in FCSd), NOM was clearly growth inhibitory like the other well known progestins already tested elsewhere, ORG2058, R5020 and MPA [3]. This progestin-induced growth inhibition could most probably be considered as an antiestrogenic response since progestins have been reported to increase the proliferation of T47-D cells [18], or to be without effect [19] when they were grown in the absence of all estrogens (DCC-stripped FCS, and phenol red-free medium).

The results of several independent experiments did not show any significant difference among progestins, while DEX had no effect, as previously shown [3]. Even NOR, a 19-nortestosterone derivative, was inhibitory on both proliferation and PgR content of T47-D cells in our studies, despite evidence that NOR, as other 19-nortestosterone derived progestins, is able to display estrogenic stimulatory effects both *in vivo* [4] and *in vitro* [21, 22]. Studies on two clones of the original T47-D cell line: one estrogen receptor rich (T47-D-A18) and one estrogen receptor negative (T47-D-C4) proved that the growth stimulation exerted by NOR (as well as by norgestrel and norethynodrel) was dependent on estrogen-receptor mediated mechanisms, which could be blocked by antiestrogens [21]. Culture conditions can explain this apparent discrepancy. The growth stimulation by 19-nortestosterone derivatives is observed in estrogen-free medium (no phenol red and DCC-stripped FCS) [21, 22]. Our present studies were performed in the presence of phenol red and unstripped decomplemented FCS, providing a sufficient estrogenic environment for optimal growth of our strain of T47-D. It should be noted that the addition of E_2 itself, even at 10^{-6} M, did not stimulate [^{3}H]thymidine incorporation.

It can be concluded that our culture conditions are not suitable to detect the estrogenic stimulation of cell proliferation by progestins of the 19-nortestosterone family, but appropriate to demonstrate progestin-induced inhibition. Further studies in an estrogen-free environment are needed to confirm that progesterone-derived compounds like MPA are devoid of any intrinsic estrogenic activity [21], to check the paradoxical estrogenic effect of R5020 at high concentration (10^{-6} M) [21] and to document in this respect the activity of the two other 19-norprogesterone derivatives studied in the present report: ORG2058 and NOM.

In keeping with the conclusion that our present culture conditions make the T47-D cells predominantly responsive to progestagenic effects, NOR like NOM and R5020 was also able to markedly reduce their PgR content. For R5020, this reduction has previously been shown to be due to both a marked increase in the rate of the receptor degradation as well as a dramatic decrease in the rate of receptor synthesis [23]. ORG2058, which we did not test on this parameter, has been demonstrated to decrease the concentration of PgR mRNA in a concentration- and time-dependent manner, the maximal effect being obtained for 10 nM with a fall in the concentration of PgR mRNA to around 20% of control [9].

Thus, our data showed that the concentration producing a half maximal decrease in PgR was far lower than the concentration producing half growth inhibition, suggesting that the mechanisms involved were differentially regulated. The lack of correlation between the sensitivity to growth inhibition by MPA and PgR levels has already been pointed out and the mechanisms related to PgR status but not to PgR concentration [3]. To sort out PgR-mediated and PgR-independent mechanisms of T47-D cells growth inhibition by progestins, studies using antiprogestins such as mifepristone (RU486) are currently being undertaken in our laboratory. In conclusion, our data on NOM are consistent with the antiproliferative effects of progestins on T47-D cells cultured in an estrogenic environment, confirming its potential therapeutic in-

terest as a progestational agent, like MPA, in the clinical management of PgR-positive breast tumours.

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