Direct Transcriptional Regulation of the Progesterone Receptor by Retinoic Acid Diminishes Progestin Responsiveness in the Breast Cancer Cell Line T-47D*

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Retinoic acid (RA) treatment of T-47D human breast cancer cells results in a rapid decrease in the concentration of progesterone receptor (PR) mRNA which causes a slow loss of cellular PR protein (Clarke, C. L., Roman, S. D., Graham, J., Koga, M., Sutherland, R. L. (1990) J. Biol. Chem. 265, 12694–12700). The mechanisms involved are unknown and this study was undertaken to determine whether the decline in PR mRNA was due to transcriptional inhibition and to evaluate the functional consequences of the RA-mediated decrease in PR. The transcription rate of the PR gene was decreased by RA, and the effect was maximal 2–3 h after treatment. Cycloheximide cotreatment was unable to relieve the inhibitory effect of RA on PR transcription suggesting that the effect was not dependent on ongoing protein synthesis. There was no effect of RA on PR mRNA half-life at the times examined (0–6 h of RA treatment). To determine the functional consequence of PR down-regulation the progestin-responsive plasmid pMSG-CAT was expressed transiently in T-47D cells which were then exposed to RA for 24 h. RA-pretreated cells were then treated with the synthetic progestin ORG 2058 and the extent of progesterin stimulation of chloramphenicol acetyltransferase (CAT) activity measured. ORG 2058 treatment resulted in an induction of CAT activity which was maximal at a progestin concentration of 1 nM. Interestingly, the ability of ORG 2058 to induce CAT activity was decreased in RA-pretreated cells. The diminished progestin responsiveness of RA-pretreated cells was confirmed in separate experiments which showed that the progestin inducibility of TGF-α mRNA was also decreased in cells treated with ORG 2058 following pretreatment with RA for 24 h. These data demonstrate that RA decreases PR mRNA concentrations by direct transcriptional inhibition, leading to decreased cellular PR concentrations. The decreased levels of PR result in impaired responsiveness to progestins and this suggests that RA derived from dietary vitamin A may have a role in modulating cellular sensitivity to progestins.

The ovarian steroid hormone progesterone plays a major role in regulating the proliferation and differentiation of a number of target tissues including the normal and neoplastic breast (1) and expression of the cellular receptor for progesterone is an important prerequisite for responsiveness to progestins. Regulation of the expression of the progesterone receptor (PR) by estrogens (2–5) and progestins (6–10) has been well defined, but there have been few, if any, studies examining the regulation of PR by other agents.

Studies from this group have recently documented the regulation of PR by the morphogen retinoic acid (RA), which decreased the concentration of PR mRNA in human breast cancer cells (11). RA has been shown to act via nuclear receptors which fall into three main groups: RAR-α, RAR-β, and RAR-γ (12–18). PR and the RARs are members of the same gene family of ligand-activated transcriptional modulators (19) and have extensive structural features in common. There is also a potential epidemiological link between steroid hormone and retinoid-mediated effects. Several studies have shown that low dietary or serum levels of β-carotene are associated with increased risk of a number of epithelial malignancies (20–22), although the involvement of RA in modulating breast cancer risk is controversial. However, retinoids have been shown to act in combination with hormones in the promotion of mammary cancer in animals (20, 22, 23). In addition to a possible involvement in carcinogenesis, retinoids can inhibit proliferation of human breast cancer cells both alone and in combination with antiestrogens (24–29). There is one report that breast tumors with high estrogen receptor levels occur in patients with high dietary retinoid levels (30).

The mechanisms underlying these observations are presently unknown, and there is only limited evidence that retinoids can directly affect steroid hormone-mediated events. RA decreases glucocorticoid receptor in squamous carcinoma cells resulting in resistance to glucocorticoid administration (31), and retinoic acid and glucocorticoids have been shown to have opposing effects on the expression of osteonectin and collagen (32).

Our previous data have shown that RA regulates the PR in human breast cancer cells primarily by a decrease in the cellular mRNA concentration followed by a gradual loss of receptor protein (11). The mechanism responsible for the decrease in mRNA concentration is unknown. The present study was designed to investigate whether the effect was mediated at a transcriptional level and if so whether the effect was direct or mediated via an intermediary protein. Further...

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† The abbreviations used are: PR, progesterone receptor; RAR, retinoic acid receptor; FCS, fetal calf serum; ORG 2058, 16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione; RA, retinoic acid; CAT, chloramphenicol acetyltransferase; kb, kilobase; TGF-α, transforming growth factor α.
more, it is not known whether the RA effect on PR results in a change in cell responsiveness to progestins. Functional interactions between RA and the thyroid hormone receptor have been described (33–36), but no such interactions have yet been described between RA and PR. Because the loss of PR protein is gradual after RA treatment, the effect of this agent may be to diminish sensitivity to progestin treatment, and therefore the responsiveness to progestins of cells pretreated with RA was investigated.

**EXPERIMENTAL PROCEDURES**

**Materials**—Materials were obtained from the sources previously listed (11). [35S]Methionine, [α-32P]UTP, and [3H]uridine were Amersham (North Ryde, Sydney, Australia); plasmids bearing the estrogen-responsive mouse mammary tumor virus long terminal repeat linked to chloramphenicol acetyltransferase (pMSG-CAT) and bacterial β-galactosidase sequences (pCH110) were obtained from Pharmacia LKB Biotechnology (North Ryde, Sydney, Australia); pSG5-hPR1 was a gift from Professor Pierre Chambon, Strasbourg, France; hRAR-α and -γ were gifts from Dr. Ronald Evans, Salk Institute, La Jolla, CA; and human fibroblast β-actin was encoded in a 2.9-kb Okayama-Berg vector (37) and human TGF-α cDNA was a 3.1-kb fragment encompassing the entire coding region for the 160 amino acid precursor of TGF-α (38), obtained from Genentech Inc. (San Francisco, CA). pSG5 was obtained from pSG5-hPR1 by excision of the hPR insert, religation of the EcoRI ends, and selection of hPR-negative clones. T-47D (39) cells were supplied by E. G. and G. Mason Research Institute, Worcester, MA and were cultured as previously described (40, 41). Cells were negative for mycoplasma contamination as determined using the Gen-Probe rapid detection system (Gen-Probe Inc., San Diego, CA).

**Isolation and Analysis of RNA**—RNA was isolated by the guanidinium isothiocyanate–cesium chloride method, and Northern analysis was carried out as described (11) except that cDNA probes were labeled by a random primer procedure using the Amersham random primer kit. To control for variation in RNA loading and transfer, membranes were stored at −20 °C until the probe had decayed and were then reprobed with β-actin cDNA. Autoradiograms were analyzed as described previously (11).

**Immunoblot Analysis of PR**—High salt cytosolic extracts of harvested cells were prepared, separated by electrophoresis, and blotted onto nitrocellulose as described previously (11). Blots were incubated with hPRa 7, a monoclonal antibody against human PR ((42) immunoglobulin fraction of hybridoma supernatant), at a final dilution of 1:1,000 and immunoreactivity was revealed colorimetrically as described (11).

**Transcriptional Analysis**—PR gene transcription was measured using the method of Greenberg and Ziff (43) as previously described (44), with modifications. Cells were plated in RPMI 1640 medium containing 5% fetal calf serum (FCS) and supplemented as described (40, 41). The medium was replaced with medium containing 1% charcoal-stripped FCS 4 days after plating and 16–24 h after medium change cells were treated with retinoic acid (10−6 M) or dimethyl sulfoxide vehicle and harvested various times thereafter. Where stated cycloheximide (20 μg/ml medium) was added at the start of treatment: control experiments showed that this concentration of cycloheximide did not inhibit inhibition of ongoing protein synthesis by 1 h after treatment (measured by [35S]methionine incorporation into trichloroacetic acid–precipitable material). Nuclei were prepared by lysis of harvested cells (43) and frozen in liquid nitrogen until required. Initiated transcripts were labeled with [α-32P]UTP as described (43) except that incubation was for 45 min. RNA was isolated by phenol/chloroform extraction, precipitated (three to four times) with 10% trichloroacetic acid until the washes were free of radioactivity, then treated with 200 mM NaOH, and run-on transcripts were quantitated using immobilized cDNA. Labeled RNA consisting of equal radioactivity/sample was hybridized at 65 °C for 36 h (43) to nitrocellulose filters onto which 5 μg each in duplicate of pSG5-hPR1, β-actin, and pSG5 cDNAs had been blotted and fixed by UV radiation. Filters were washed (43), dried, and exposed to x-ray film. Autoradiograms were analyzed densitometrically, and the mean height of duplicate PR RNA spots on filters bound to PR mRNA treated samples was normalized for the mean heights of the duplicate β-actin signals on the same filter, then expressed as a percentage of the similarly normalized PR RNA signal on control filters.

**Labeling of RNA with [3H]Uridine**—PR mRNA half-life was determined using the pulse-chase technique (44) with modifications. Cells were incubated in the presence of 200 μCi/ml [3H]uridine (39 Ci/mmol) for 5 h and the medium replaced with medium containing 5 mM unlabeled uridine and 2.5 mM cytidine in the presence of 10−6 M RA or vehicle. Cells were preincubated 1, 2, and 4 h prior to the medium change. Total cellular RNA was isolated as described above for transcriptional analysis. The yield of RNA was similar in treated and control groups at all times. Tritiated PR mRNA levels in each RNA sample were determined using the membrane-bound excess cDNA technique (45), and filters were prepared as described for transcriptional analysis. Membranes were prehybridized overnight at 50 °C, and then equal amounts of RNA/time point were added and filters hybridized as described previously (6). Membranes were washed and treated with ribonuclease A (43). Radioactivity in the excised membrane slots was determined by liquid scintillation. Specific radioactivity was determined by subtracting the radioactivity in pSG5 slots from the radioactivity in pSG5-hPR1 or β-actin slots.

**Gene Transfection into T-47D Cells**—Cells were plated into 150-mm dishes (2.5–5.0 × 104 cells/dish) in RPMI 1640 medium containing 5% FCS 3 days prior to transfection. On the morning of transfection medium was changed to Dulbecco’s modified Eagle’s medium, PH 7.3–7.4, containing 5% FCS, and cells were cotransfected, using the calcium phosphate precipitation method (46), with pMSG-CAT (40 μg/flask) and pCH110 (10–20 μg/flask). Cells were subjected to osmotic shock using 15% glycerol (45 μl) 4 h after transfection and exposure to the DNA continued for 18–24 h thereafter. Cells were harvested, mixed, and replated into multiwell plates (1–3 × 105 cells/well) to reduce variations due to interassay differences in transfection efficiencies. When the cells had adhered to the substrate (3–6 h after plating) RA (10−6 M) or vehicle was added and cells were further treated 3, 6, or 24 h thereafter with ORG 2058 at the indicated concentrations. Cells were harvested, counted, and lysed in 0.25 M Tris/HCl buffer, pH 7.8, by freeze thawing (three times). Aliquots were removed from the lysate for measurement of cell-free β-galactosidase activity (46) and the lysates heated at 65 °C for 10 min. After centrifugation, an aliquot was removed for measurement of CAT activity by a non-chromatographic method (47). Protein determination was by the Biocat method. CAT activity was normalized for cell-free β-galactosidase activity. Transfection efficiencies and the effect of treatment on pCH110 expression were estimated by counting β-galactosidase-positive cells revealed on cell monolayers using the histochemical indolyl method (48, 49). Transfection efficiencies between experiments typically varied between 0.1 and 0.6%.

**RESULTS**

**Retinoic Acid Decreased the Transcription Rate of the PR Gene**—Treatment of T-47D cells with 10−6 M RA resulted in a decrease in the transcription rate of the PR gene, which was seen 1 h after and persisted for at least 3 h after treatment (Fig. 1). This decrease in transcription rate preceded the previously demonstrated fall in PR mRNA levels (Fig. 1 and Ref. 11). The effect of RA on PR gene transcription persisted in the presence of cycloheximide, suggesting that it was independent of ongoing protein synthesis (Fig. 2). The decrease in PR mRNA levels is consistent with the effect resulting primarily from a transcriptional event. The transcription rate recovered partially at 6 h (Fig. 1) although PR mRNA levels remained depressed for at least 36–48 h (11). The effect of RA on PR gene transcription persisted in the presence of cycloheximide, suggesting that it was independent of ongoing protein synthesis (Fig. 2). The increase in β-actin transcription rate upon cycloheximide exposure has been described previously (50).

**PR mRNA Half-life Was Unaffected by RA Treatment**—In order to verify whether the inhibitory effect of RA on PR mRNA was due primarily to inhibition of transcription or whether RA also affected PR mRNA half-life, total cellular RNA was isolated from cells which had been pulse-labeled using [3H]uridine, then treated with 10−6 M RA or vehicle for 6 h. The radioactivity in PR and actin RNA was measured over time. PR mRNA decreased rapidly and reached background levels within 6 h (Fig. 3). The rate of the decrease in
Fig. 1. Effect of RA and ORG 2058 on PR gene transcription. Nuclei from $10^{-8}$ M RA (●) or vehicle-treated cells ($8 \times 10^6$ cells/point) were incubated with [$\alpha$-$^{32}$P]UTP and labeled RNA was quantitated using immobilized cDNA. PR RNA was normalized as described under "Experimental Procedures" and expressed as a percentage of PR in vehicle-treated controls. Symbols represent the mean ± S.E. of 3 (2 h) or range ± range of 2 (1 and 6 h) separate experiments. The 2-h data were a single determination. The range/error bars for the 3-h RA-treated samples did not exceed the size of the symbol. The effect of $10^{-8}$ M RA on PR mRNA levels (●) is shown for comparison and was redrawn from Ref. 11 to show 0–6 h only.

Fig. 2. Effect of cycloheximide treatment on PR gene transcription. Labeled PR RNA transcripts isolated from cells treated for 1 h with $10^{-8}$ M RA or vehicle in the presence or absence of cycloheximide (20 μg/ml) were quantitated using immobilized cDNA. Autoradiograms were scanned densitometrically, PR was normalized as described under "Experimental Procedures" and expressed as a percentage of vehicle-treated controls. Open bars, control; hatched bars, RA treated.

PR mRNA concentration was unaffected by RA, and the radioactivity in β-actin mRNA did not change appreciably over the time course examined.

RAR-α and -γ mRNAs Are Present in T-47D Cells—T-47D cells treated with increasing concentrations of RA were examined for the presence and regulation of RA receptors. RAR-α was detected as a doublet of 2.7 and 3.4 kb, and RAR-γ was detectable as a single mRNA species of 3.4 kb (Fig. 4). Treatment of T-47D cells with increasing concentrations of RA for either 6 h (not shown) or 48 h (Fig. 4) had no effect on the mRNA levels of either receptor. RAR-β mRNA was barely detectable in T-47D cells and was increased to low levels after 6 or 48 h of treatment with $10^{-8}$ M or $10^{-9}$ M RA (not shown).

RA Treatment Causes Diminished Progesterin Responsiveness of T-47D Cells—In order to examine the functional consequences of RA-mediated PR loss, the effect of RA on endogenous ORG 2058-inducible events was examined. TGF-α was chosen as an endogenous progesterin-sensitive end point because its mRNA levels are increased by progestins in breast cancer cells (51–53). T-47D cells were pretreated with RA for 24 h at which time PR protein levels were around 50% (Fig. 5, lane 2) of vehicle-pretreated controls (Fig. 5, lane 1) as previously described (11). Cells were then treated for 9 h with ORG 2058, which increased the size of the receptor proteins as previously described (54–59) in both vehicle-pretreated and RA-pretreated samples (Fig. 5, lanes 4 and 6). In samples which had not been treated with ORG 2058, the concentration of PR in the RA-pretreated sample at 9 h (Fig. 5, lane 5) was
Fig. 5. Immunoblot analysis of PR. T-47D cells were grown in RPMI 1640 medium containing 5% FCS for 4 days then changed to medium containing 1% FCS 24 h prior to treatment with 10⁻⁶ M RA (lanes 2, 5, and 6) or vehicle (lanes 1, 3, and 4). After 24 h of exposure to RA (time 0; lane 1, vehicle pretreated and lane 2, RA pretreated), cells were treated with 10 nM ORG 2058 (lane 4, vehicle pretreated and lane 6, RA pretreated) or vehicle (lane 3, vehicle pretreated and lane 5, RA pretreated) and harvested 9 h thereafter. High salt cytosol extracts (200 μg/lane) were analyzed by immunoblotting. Arrows indicate the 116- and 83-kDa immunoreactive PR proteins.

Fig. 6. Effect of RA pretreatment on ability of ORG 2058 to induce TGF-α mRNA. T-47D cells were grown in RPMI 1640 medium containing 5% FCS for 4 days, then changed to medium containing 1% FCS 24 h prior to treatment with 10⁻⁶ M RA (lanes 2 and 7-10) or vehicle (lanes 1 and 3-6). After 24 h (time 0, lanes 1 and 2), cells were treated with 1 (lanes 4 and 8), 10 (lanes 5 and 9) or 100 (lanes 6 and 10) nM ORG 2058 and harvested 9 h thereafter. Lanes 3 and 7 represent ORG 2058-untreated samples at 9 h. Total RNA was probed with TGF-α cDNA and subsequently with β-actin to verify loading and transfer. Autoradiograms were scanned densitometrically and the peak heights expressed in arbitrary units (AU) for control (open bars) and RA- (hatched bars) pretreated samples.

Further diminished relative to zero time (Fig. 5, lane 2), consistent with a continuing slow decline in immunoreactive PR levels upon prolonged RA treatment (11), whereas PR levels in vehicle-pretreated controls remained the same (Fig. 5, compare lane 3 with lane 1). The concentration of TGF-α RNA was estimated 9 h after progesterin treatment. ORG 2058 treatment caused a 2-2.5-fold increase in the abundance of this mRNA species (Fig. 6), and the ORG 2058 effect was dose-dependent as previously shown (51). RA itself was ineffective in modulating TGF-α mRNA levels, but the magnitude of the ORG 2058 mediated increase was blunted in cells which had been pretreated with RA for 24 h (Fig. 6). The RA-mediated decrease in ORG 2058 induction of TGF-α mRNA was seen at all the ORG 2058 concentrations tested, not only at 9 h (Fig. 6) but also at 3 and 6 h (not shown) after treatment. RA pretreatment also decreased the ability of ORG 2058 to induce c-myc mRNA (not shown). Overall, however, RA had only modest effects in decreasing progesterin sensitivity of endogenous progesterin-sensitive mRNAs. This could be due to a number of factors including the still unknown mechanism through which ORG 2058 induces these mRNAs. If the ORG 2058 effect is indirect, the PR-mediated component of the response may be minor and RA-mediated decreases in PR have little effect on the magnitude of the outcome.

In order to address more directly the functional consequences of RA-mediated decreases in PR, the progesterin-responsive plasmid pMSG-CAT was transfected into T-47D cells. This plasmid contains the long terminal repeat of the mouse mammary tumor virus, and this has several progesterin-responsive elements with which PR has been shown to interact directly (60-63). ORG 2058 treatment of T-47D cells transiently expressing pMSG-CAT resulted in induction of CAT activity which was essentially maximal 24-48 h after (Fig. 7, inset) and persisted at least until 68 h after ORG 2058 treatment, which was equivalent to a total time of 96-h posttransfection. The maximal induction achieved was proportional to the quantity of plasmid transfected (not shown). The effect of ORG 2058 on CAT induction was maximal at a concentration of 1 nM (Fig. 7). The progestin R5020 and the synthetic androgen R1881 (each 10 nM) also induced CAT activity maximally whereas 17β-estradiol and dexamethasone were without effect (not shown). The inability of glucocorticoids to induce MMTV-CAT activity in T-47D cells has been shown previously (60, 62, 63) and is attributable to the low glucocorticoid receptor concentration in these cells (64, 65). No effect of RA or ORG 2058 at any concentration was noted on the expression of pCH110 (not shown).

T-47D cells were pretreated with RA prior to treatment with ORG 2058 in order to determine whether RA-mediated loss of PR protein would result in a diminished ability of ORG 2058 to induce CAT activity. RA pretreatment was for 24 h, which corresponded to the time needed for RA to cause essentially maximal loss of receptor protein (11). In cells pretreated with vehicle for 24 h, ORG 2058 treatment increased CAT activity 7.3-fold at 0.1 nM and 9.5-fold at 10 nM (Fig. 8A). However, in cells which had been exposed to RA for 24 h and consequently had lower PR levels (11), the induction of CAT activity was diminished to 3.1-fold at 0.1 nM and 6.4-fold at 10 nM ORG 2058 (Fig. 8A). The decreased ability of ORG 2058 to induce CAT activity was more pro-
Transcriptional Regulation of Progesterone Receptor

Thereafter (11). It is likely that transcriptional inhibition was transient, being maximally decreased a short time after treatment but recovering to close to control levels at longer times. This effect on transcription is comparable with the known effect of RA on PR mRNA levels. RA-mediated PR mRNA loss is maximal 3–6 h after treatment, and the decreased level is sustained at least until 24–48 h thereafter (11). It is likely that transcriptional inhibition was pronounced at 0.1 nM, a concentration which was submaximal for CAT induction (Fig. 7).

The ability of ORG 2058 to induce CAT activity was examined also in samples which had been RA pretreated for either 3 or 6 h. This time frame was chosen as it precedes the fall in PR protein seen upon RA exposure (11). There was no difference in the induction of CAT in the 3/6-h vehicle (9-fold) and RA (8.9-fold) pretreated samples by 10 nM (Fig. S8F) or at lower ORG 2058 concentrations (0.1, 1 nM, not shown).

**DISCUSSION**

This study has shown that RA was able to decrease the transcription rate of the PR gene. The decrease in transcription rate was transient, being maximally decreased a short time (3 h) after treatment but recovering to close to control levels at longer times. This effect on transcription is comparable with the known effect of RA on PR mRNA levels. RA-mediated PR mRNA loss is maximal 3–6 h after treatment, and the decreased level is sustained at least until 24–48 h thereafter (11). It is likely that transcriptional inhibition was sufficient to account for the decline in mRNA levels up to 6 h after treatment, as no effect of RA on the half-life of PR mRNA was noted during this time. However, the sustained lowering in PR mRNA levels beyond 6 h of treatment takes place in the face of an apparent recovery in transcription rate documented in this study and suggests that although the rapid fall in PR mRNA levels can be attributed to a transcriptional event in the acute phase (0–6 h), it is possible that posttranscriptional mechanisms come into play thereafter. Posttranscriptional regulation of RNA processing by retinoids has been described (reviewed in Ref. 20). Furthermore, posttranscriptional events have been invoked to explain the decrease in estrogen receptor mRNA levels after estrogen treatment, which causes only transient decreases in the transcription rate of the estrogen receptor gene, in MCF-7 cells (66).

The rapidity of the effect of RA on transcription suggested the possibility that it was direct. Coincubation of cycloheximide and RA under conditions where new protein synthesis had been inhibited by over 90% failed to abrogate the transcriptional inhibition, evidence that no intermediate protein synthesis was required. This suggests that the effects of RA on PR may be mediated by direct interaction with PR promoter sequences. It is intriguing to note that the decrease in PR mRNA levels mediated by RA never exceeds 50%, in contrast with the profound decrease caused by progesterin treatment (6–10). One explanation could be that RA affects only one of the two known PR promoters (67). Alternate transcription start sites and polyadenylation signals have been shown (67) to result in the five PR mRNA species described previously (8–10, 68) in which case preferential loss of one or more PR mRNA species may be detectable. Presently there is no evidence for such preferential loss upon RA treatment, although analysis of total RNA, in which the PR mRNA species smaller than 6 kb fail to be fully resolved around the 18 S and 28 S ribosomal RNA subunits, probably precludes clear demonstration of such a phenomenon. However, there is evidence that the ratio of the A and B PR proteins remains the same at all times after RA treatment (not shown).

The RA effects on PR are likely to be mediated by one of the family of recently described RAR (12–18). Two classes of RAR, the α and γ, were detectable in T-47D cells as previously described (16). There was no effect of RA on RAR-α at any concentration tested which is consistent with what has been described in human hepatoma cells and for the murine receptor in F9 cells (18, 69). There was also no effect of RA on RAR-γ in contrast with the murine homologue which is decreased by RA treatment (18). RAR-β was barely detectable in T-47D cells as previously shown (16) but was inducible to a low level at high RA concentrations, consistent with the presence of RA-responsive elements in the upstream region of the receptor gene (70, 71). It is not yet known which of the RA receptors is responsible for regulating PR gene transcription. However, the fact that the α and γ species fail to be regulated by RA treatment suggests that their continued presence in the face of high RA levels may allow a sustained effect of RA acting through either RAR on lowering PR levels.

The consequences of RA treatment on progesterin reponsiveness were evaluated to assess the potential physiological significance of the data presented in this study. Transfection into T-47D cells of a reporter plasmid containing the progesterin-responsive long terminal repeat of the mouse mammary tumor virus (60) resulted in rapid and sensitive induction of CAT activity upon progesterin treatment as previously described (60–63). Pretreatment of transfected cells with RA for short periods of time during which PR protein levels were unaffected by RA had no effect on the ability of progesterin to...
induce CAT activity. However, when the RA pretreatment was prolonged, producing a maximal decrease in PR protein levels, the cell responsiveness to progesterin was markedly reduced. The induction of CAT activity by 0.1 nM ORG 2058 in RA-pretreated cells was 40–50% of that noted in vehicle-pretreated cells. The magnitude of this effect is of the same order as the magnitude of the RA-mediated loss of PR protein (11). The effect of RA pretreatment was more evident at concentrations of ORG 2058 which were submaximal for CAT induction and therefore less likely to be blunted by transcription factor limitations which may occur at high activated receptor concentrations (72).

This study raises the question of whether vitamin A-derived retinoids may play a part in the modulation of steroid hormone-mediated action in breast cancer. Retinoids are known antiproliferative and differentiation agents in a wide range of tissue and cell types (20–22) and can inhibit proliferation of human breast cancer cells at similar concentrations to those used in this study (24–29). However, the effects of RA on PR documented in this study are unlikely to be simply secondary to effects of RA on proliferation, as the proliferation status of T-47D cells has little or no effect on cellular PR concentrations (not shown).

A relationship between retinoid intake or serum levels of retinoids and breast cancer incidence has been proposed but has been difficult to establish. Case control and prospective studies have both supported (73, 74) and refuted (75–77) the hypothesis that low dietary or serum β-carotene levels are associated with an increased risk of breast cancer. Furthermore, despite the link postulated in some other cancers between low β-carotene levels and increased risk (20–22), few if any studies have found such a link with retinol, perhaps because serum levels of carotenoids vary with nutritional intake, whereas homeostatic mechanisms maintain serum retinol levels within a defined range (21). It has yet to be demonstrated, in addition, whether the protective effects of β-carotene are due to their antioxidative properties or to local metabolism to retinol or retinoic acid (21), nor has the extent of tissue retinol or retinoic acid conversion from β-carotene been determined.

Although the role of retinoids in breast cancer incidence and/or progression has yet to be clarified, this study and in vitro studies cited above, which have examined the effects of retinoids directly on molecular events, have provided evidence for a functional association between retinoid and steroid hormone-mediated events in breast cancer cells. This study has demonstrated that RA treatment decreases PR gene transcription and results in reduced cell sensitivity to progestins. Whether this reduced sensitivity could result in diminished ability of breast cancers to respond to progesterin therapy, or whether loss of sensitivity to progestins during a critical period of breast differentiation during puberty may alter normal breast cell susceptibility to carcinogenic insult is presently unknown.

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