Antiestrogen Action of 2-Hydroxyestrone on MCF-7 Human Breast Cancer Cells*

Jill Schneider, Martin M. Huh, H. LeonBradlow, and Jack Fishman†

From The Rockefeller University, New York, New York10021

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The estrogen responsive human breast cancer MCF-7 cell culture was examined for its response to 2-hydroxyestrone a principal metabolite of estradiol. Addition of 2-hydroxyestrone to the cell cultures in concentration of 10-6-10-4 M had no effect on cell growth and proliferation because of rapid O-methylation of the catechol estrogen by catechol O-methyltransferase which is highly active in these cells. In the presence of quinolinizarin, a potent catechol O-methyltransferase inhibitor which reduces the O-methylation of the steroid, 10-7 M and 10-6 M 2-hydroxyestrone markedly suppresses the growth and proliferation of the cells. The tumor cell growth-inhibitory action of the catechol estrogen was neutralized by the presence of 10-6 M estradiol. The catechol estrogen inhibition of cell growth is not observed in the estrogen receptor-negative human breast cancer cell lines MDA-MB-231 and MDA-MB-330 providing evidence that the inhibition is specific and is estrogen receptor-mediated. In contrast, the 16a-hydroxylated metabolites of estradiol, estriol and 16a-hydroxyestrone, are effective stimulators of MCF-7 cell proliferation with the latter exhibiting potency in excess of that expected from its estrogen receptor affinity. The present results represent the first observation of a specific receptor-mediated antiestrogenic action of 2-hydroxyestrone and suggest that the physiological regulation of the agonist activity of the primary estrogen may involve in situ generation of catechol estrogen.

The catechol estrogens are quantitatively the most important metabolic products of the female sex hormone estradiol (1). Although their exact physiological role in the mediation and/or modification of the hormonal actions of estrogen is still the subject of much study, they have already been shown to exert numerous biological effects in both peripheral as well as central estrogen target tissues. These effects can be characterized as reflecting either estrogen agonist or antagonist actions with the most prolific catechol estrogen, 2-hydroxyestrone, exhibiting minimal agonist activity but providing evidence of estrogen antagonist action in neuroendocrine events (2-4). The mechanisms underlying the action of the catechol estrogen are not clearly understood, although data on their interaction with the estrogen cytosolic receptor (5, 6) and its subsequent translocation into the nucleus (7, 8) indicate that at least some of their actions may involve competition with the primary estrogen for the receptor. The catechol estrogen may also act via their interaction with the catecholaminergic system. They have been reported to bind specifically to dopamine and alpha-adrenergic receptors in the brain and pituitary (9, 10). They inhibit tyrosine hydroxylase, the rate-limiting enzyme of catecholamine biosynthesis (11, 12) and can thus decrease catecholamine content. Conversely they are highly effective competitive inhibitors of the metabolism of catecholamines by catechol O-methyltransferase and would thus decrease the turnover time of these biogenic amines (13, 14). Indeed, it is now apparent that catechol estrogen administered in vivo are rapidly metabolized by the action of tissue and erythrocyte catechol O-methyltransferase resulting in very high metabolic clearance rates for these compounds (15-17). This feature of their biology makes it difficult to define their intrinsic physiological properties by in vivo administration.

In the present study we have used the hormone-dependent human breast cancer cell line MCF-7 in tissue culture as a system for the study of the biological effects of 2-hydroxyestrone, under conditions where its further metabolism could be controlled, and its activity in peripheral estrogen target sites be evaluated. Our results indicate that the catechol estrogen behaves as an antiestrogen in suppressing the tumor cell proliferation both under control and estradiol stimulatory conditions. This activity of the catechol estrogen becomes apparent in the presence of the inhibitors of catechol O-methyltransferase an enzyme whose activity is greatly increased in this tumor tissue.

EXPERIMENTAL PROCEDURES

Materials—Human breast cancer cell lines MCF-7, MDA-MB-231, and MDA-MB-330 were obtained from the Breast Cancer Human Cell Culture Bank of the National Cancer Institute (maintained by EG & G Mason Research Institute, Worcester, MA). 2-[6,7-3H]hydroxyestrone (specific activity, 40-60 Ci/mmole), purchased from New England Nuclear, was purified by thick layer chromatography as described (18) to ensure the absence of any contamination by other steroids, particularly estradiol. Estradiol and estriol were obtained from Steroids Inc. (Wilkin, NH), 16a-hydroxyestrone from Sigma, and quinolinizarin from Aldrich Chemical Co. All steroids were checked for purity by chromatographic and spectral criteria, and were purified to homogeneity as required. RPMI 1640 medium, fetal bovine serum, penicillin-streptomycin suspension (10,000 units and 10,000 μg, respectively/ml), mycostatin suspension (10,000 units/ml), trypsin-EDTA (1X), Eagle's balanced salt solution, and Eagle's balanced salt solution without Mg²⁺ and Ca²⁺ were obtained from Gibco Labs (Grand Island, NY). Sterilization filters (0.45 μm and 250 ml or 500 ml capacity) were purchased from Nalge Co. (Rochester, NY).

Cell Culture—The MCF-7 cells were subcultured in T-75 flasks by daily changes of the "passage" medium (50 ml) consisting of RPMI 1640 supplemented with fetal bovine serum (10%, v/v), which were maintained in a humidified chamber at 37 °C with 5% CO₂ and 95% air. The cells were harvested at confluency by trypsinization and suspended at a cell density of 200,000 to 300,000 cells/ml in a small volume of the growth medium to be used.

In a typical experiment in which the effects of hormones on the
cell growth were to be examined, aliquots of the cell suspension containing about 20,000 to 30,000 cells were replicately plated in dishes (60 mm in diameter) containing 3 ml of the growth medium consisting of RPMI 1640 supplemented with charcoal-stripped fetal bovine serum (5%, v/v) and of penicillin-streptomycin (10 units and 10 μg, respectively/ml of medium) and mycostatin (2.5 units/ml) and incubated at 37°C in the humidified chamber. On day 1 when the cells adhered firmly to the bottom surface of the dishes, the medium was aspirated off and the cells then challenged by hormones; groups of replicate dishes received 3 ml of fresh growth medium containing a hormone at the indicated concentrations. Another group of replicate dishes which received only the vehicle served as the nonhormone-treated controls. All dishes, unless otherwise stated, also received quinalizarin (dissolved in dimethyl sulfoxide), a potent inhibitor of catechol O-methyltransferase (19), at a concentration of 10⁻⁶ M, a concentration which does not exert any effect on cell growth but is effective in inhibiting the enzyme. Each group received a change of the fresh growth medium (3 ml) every 24 h unless otherwise noted, at which time the hormone-treated group received the hormone at the indicated levels and the control group the vehicle. At various time intervals, i.e. 24 h, triplicate dishes were withdrawn for cell counting; after removal of the medium by aspiration, the adhered cells were washed twice with 1 ml of Eagle’s balanced salt solution without Ca²⁺ and Mg²⁺ and dislodged from the dish by trypsinization involving the incubation with 0.5 ml of trypsin–EDTA solution (1X) for 5 min at 37°C, after which the protease reaction was stopped by adding 2.5 ml of a “stop” medium consisting of RPMI 1640 and 5% fetal bovine serum. The cells were then collected by centrifugation with subsequent suspension in the medium. An aliquot of the suspension was subjected to cell counting using a hematocytometer. In the present study we focused on cell proliferation as an index of estrogen response subjected to cell counting using a hematocytometer. In the present study we focused on cell proliferation as an index of estrogen response subjected to cell counting using a hematocytometer.

The estrogen-independent human breast cancer cell lines MDA-MB-231 and MDA-MB-330 were cultured in a manner identical with that described above for MCF-7.

**RESULTS**

**Rapid Metabolism of 2-Hydroxyestrone by O-Methylation**—Our initial attempt to demonstrate the effect of 2-hydroxyestrone on the tumor cell growth and proliferation of a hormone-dependent human breast cancer cell line, MCF-7, in cell culture, met with a lack of response. Analysis of the metabolites of the catechol estrogen, however, revealed that the failure to observe any effect was due to rapid metabolism of the steroid via O-methylation by catechol O-methyltransferase which was highly active in these cells. As shown in Fig. 1, 2-hydroxyestrone, when presented at a concentration of 10⁻⁷ M to the 600,000 cells growing exponentially in RPMI 1640 medium supplemented with 5% calf bovine serum stripped of endogenous estrogen, was rapidly metabolized to O-methylated products as analyzed by thin layer chromatography. The metabolism is essentially complete in 1–2 h with 30 to 40% of the total dose being O-methylated and remaining unchanged subsequently for up to 48 h. The remainder of the dose is retained intracellularly; the nature of the cell-retained material was not investigated. It should be noted that 2-hydroxyestrone appears to undergo a two-stage metabolism in this system; an early phase conversion to unidentified polar metabolites (Fig. 1) and a subsequent biotransformation to 2-methoxyestrone. In any event no intact 2-hydroxyestrone is

![FIG. 1. Rapid metabolism of 2-hydroxyestrone (2-OHE₁) in the human breast cancer cell line MCF-7 and identification of its metabolites by thin layer chromatography. The cells were grown as described under “Experimental Procedures” except that the medium consisted of RPMI 1640 containing 5% fetal bovine serum and 2-[6,7-³H]hydroxyestrone was added to the cells at a final concentration of 5 × 10⁻⁸ M on day 6 when the cells were growing exponentially. At various time intervals indicated, the medium was withdrawn, processed, and analyzed for the metabolites by thin layer chromatography as described under “Experimental Procedures.” 2MeOE₁, 2-methoxyestrone.](http://www.jbc.org/)

1 The calf bovine serum was stripped of endogenous estrogens by treatment with charcoal (1 g/100 ml of serum) with stirring in a dark cold room for 24 h, after which the serum was recovered by filtering under vacuum on acid-washed Celite paper, followed by sterilization filter. Complete removal of the unconjugated steroids was verified by a test in which an exogenously added labeled steroid was completely removed by the identical treatment. It is known however (28) that this treatment does not result in complete removal of estrogen sulfates which could serve as sources of estrogen stimulus upon deconjugation by the tissue.
becomes apparent in the presence of the potent catechol estrogen and does inhibit the enzyme (Fig. 2). Under these conditions, the rate of unstimulated control cells, with a maximal stimulation, is discernible in the medium approximately 1 h after its introduction.

Antiestrogenic Activity of 2-Hydroxyestrone in the Presence of Catechol O-Methyltransferase Inhibitors—The response of the human breast cancer cell MCF-7 to the catechol estrogen becomes apparent in the presence of the potent catechol O-methyltransferase inhibitor, quinalizarin (19), at a concentration of 10^{-8} M which does not cause cytotoxicity to the cells but does inhibit the enzyme (Fig. 2). Under these conditions, 2-hydroxyestrone at a concentration of 10^{-8} M or 10^{-7} M effectively inhibited cell proliferation of the MCF-7 cells. Under identical conditions estradiol at as little as 10^{-11} M stimulated the tumor cell growth approximately 2-fold over the rate of unstimulated control cells, with a maximal stimulation occurring at a concentration in the range of 10^{-8} to 10^{-9} M.

Reversal by Estradiol of 2-Hydroxyestrone-induced Growth Inhibition—The tumor cell growth inhibition elicited by the 10^{-7} M 2-hydroxyestrone in the presence of the catechol O-methyltransferase inhibition (i.e. quinalizarin at 10^{-8} M) was overcome by the simultaneous challenge on day 1 by estradiol (19) at a concentration in the range of 10^{-10} to 10^{-9} M (Fig. 3), suggesting that the 2-hydroxyestrone and the primary estrogen compete with each other for the cellular site(s) of action which is presumably the estrogen receptor. This result is consistent with the notion that the tumor growth-inhibitory action of 2-hydroxyestrone is mediated by the estrogen receptor.

Inability of Estradiol to Rescue the 2-Hydroxyestrone-inhibited MCF-7 Cells—In contrast to the result of the experiment in which the simultaneous challenge on day 1 of the primary estrogen and the 2-hydroxyestrone results in the reversal of the catechol estrogen inhibition, the addition of the former (at a concentration of 10^{-9} or 10^{-8} M) on day 4 under identical condition failed to rescue the catechol estrogen-inhibited cells (Fig. 4), suggesting that in the absence of estradiol during the

![Graph](http://www.jbc.org/)

**Fig. 2 (left).** Tumor cell growth inhibition of a hormone-dependent human breast cancer cell line MCF-7 by the catechol estrogen, 2-hydroxyestrone. The cells were plated on day 0 as described under “Experimental Procedures” and exposed to the hormones on day 1 (as indicated by an arrow) by changing the medium to the growth medium containing each hormone at the indicated concentrations. Quinalizarin was present in the medium for all hormone-treated cell groups at a concentration of 10^{-8} M to inhibit catechol O-methyltransferase (19). The hormone-untreated control and the catechol O-methyltransferase inhibitor control cell groups received the medium containing the vehicle alone and the inhibitor alone, respectively. Media changes were made every other day (on days 3 and 5). At various time intervals, i.e. 24 h, triplicate dishes in each group were removed and subjected to cell counting as described under “Experimental Procedures.” The points represent mean of triplicate samples. Hormone challenges were: •—•, 2-hydroxyestrone, 10^{-7} M; ■—■, none (the vehicle alone); x—x, none (quininalizin, 10^{-8} M); ◼—◼, 10^{-10} M estradiol; and △—△, 10^{-9} M estradiol.

**Fig. 3 (center).** Reversal of the estrogen receptor-induced growth inhibition of MCF-7 cells by the primary estrogen estradiol. Groups of replicate dishes containing an equal number of the cells, plated on day 0 under the conditions described under “Experimental Procedures,” were challenged by the catechol estrogen alone, by estradiol alone, or by the catechol estrogen and estradiol simultaneously in combination at the indicated concentrations on day 1 as indicated by the arrow. Control groups received no hormone but the vehicle and all groups also received quinalizarin at a concentration of 10^{-8} M. Media were changed every other day (on days 3 and 5) when each group received its respective hormones singly or in combination, together with the catechol O-methyltransferase inhibitor. At the indicated time intervals triplicate dishes were withdrawn and subjected to cell counting as described under “Experimental Procedures.” Hormone challenges were: •—•, 10^{-7} M 2-hydroxyestrone; ■—■, none; ◼—◼, 10^{-7} M 2-hydroxyestrone plus 10^{-11} M estradiol; ◼—◼, 10^{-10} M estradiol alone; △—△, 10^{-7} M 2-hydroxyestrone plus 10^{-8} M estradiol; and △—△, 10^{-9} M estradiol alone.

**Fig. 4 (right).** Irreversibility of the action of the catechol estrogen on the cell growth and proliferation of MCF-7 cells. Groups of replicately plated cells were exposed daily to 2-hydroxyestrone (20HE1) at the concentration of 10^{-7} M. A group of control cells received the vehicle alone. The catechol estrogen-treated groups were then challenged by estradiol (E2) on day 4 (as indicated by the arrow) at either 10^{-11} or 10^{-9} M, both concentrations adequate to normally stimulate the cell growth and proliferation of the estrogen-responsive tumor cells (Fig. 3). Quinalizarin was present at a concentration of 10^{-8} M in all dishes. Media were changed daily. At various time intervals after estradiol treatment on day 4, triplicate dishes were removed for cell counting as described under “Experimental Procedures” □—□, hormone-untreated control cells; ◼—◼, cells treated with 2-hydroxyestrone (10^{-9} M) throughout the experiment; ○—○, cells treated with 2-hydroxyestrone (10^{-7} M) until day 4 and then simultaneously challenged to both the catechol estrone (10^{-7} M) and estradiol (10^{-11} M) thereafter in an attempt to rescue the cells; and △—△, cells treated with 2-hydroxyestrone (10^{-9} M) until day 4 and then simultaneously challenged to both the catechol estrone (10^{-7} M) and estradiol (10^{-9} M) thereafter in an attempt to rescue the cells.
early days of cell growth the catechol estrogen effects an irreversible alteration of the estrogen-responsiveness of the MCF-7 cells. The irreversible action of the catechol estrogen may also involve estrogen receptor-linked events.

Effects of 2-Hydroxyestrone on the Tumor Cell Growth of Estrogen Receptor-negative Human Breast Cancer Cells—An additional line of evidence supporting the notion that the estrogen receptor-mediated action of 2-hydroxyestrone is brought about by estrogen receptor-mediated events stems from the results of the experiments (Fig. 5) indicating that estrogen receptor-positive human breast cancer cell lines, such as MDA-MB-231 and MDA-MB-330, grown under conditions similar to those for the MCF-7 cells, do not exhibit any growth-inhibitory response to 2-hydroxyestrone at a concentration of \(10^{-7}\) M (Fig. 5b) in the presence of catechol 0-methyltransferase inhibitors. Under identical conditions the estrogen receptor-positive MCF-7 cell exhibited the growth-inhibitory action of 2-hydroxyestrone as well as the growth-stimulatory response to estradiol (Fig. 5a). Both of the estrogen receptor-negative cancer cell lines did not respond to estradiol even at as high as \(10^{-8}\) M (Fig. 5b), confirming their estrogen-unresponsiveness of both cell lines. These results, taken together, suggest that the action of 2-hydroxyestrone on the MCF-7 cells is mediated by the estrogen receptor-related events.

Effects of 16a-Hydroxy Metabolites of Estradiol on the Cell Growth of MCF-7—We examined 16a-hydroxyestrone and estriol, the estradiol metabolites formed in the 16a-hydroxylative pathway, for their ability to affect the growth of the MCF-7 cells. The results, shown in Fig. 6, indicate that these metabolites, unlike the 2-hydroxylated derivatives, promote the cell proliferation of the breast cancer cells at concentrations in the range of \(10^{-7}\) to \(10^{-8}\) M in the potency order of estradiol > 16a-hydroxyestrone > estriol. The agonistic (estrogenic) potency order of these estradiol metabolites observed in the human breast cancer MCF-7 cells is not representative of their relative affinity for the estrogen receptor since the very much more potent 16a-hydroxyestrone is a less effective ligand for the receptor than estriol (22).

DISCUSSION

The data presented indicate that 2-hydroxyestrone, the most ubiquitous catechol estrogen, possesses a growth-inhibitory activity on an estrogen-dependent human breast cancer cell line MCF-7. The evidence that this effect of the catechol estrogen is mediated through the estrogen-receptor is 2-fold. Firstly, the effect is antagonized by physiological levels of the primary estrogen estradiol in the range of \(10^{-11}\) to \(10^{-9}\) M (Fig. 3). Secondly, the growth-inhibitory effect of the catechol estrogen is not observed in the estrogen receptor-positive human breast cancer cell lines MDA-MB-231 and MDA-MB-330 (Fig. 5b) under conditions where the estrogen receptor-positive cell line MCF-7 responds to both the catechol estrogen and the primary estrogen (Fig. 5a). These results, together with the data on the in situ formation of the catechol estrogen from estradiol in human breast cancer tissues (23, 24) and others (2), suggest that the catechol estrogen may function as an endogenous antiestrogen, suberving a physiological mechanism by which the catechol estrogen normally exerts a negative regulatory control to oppose the agonist action of the primary estrogen.

Our data on the metabolism of the catechol estrogen in the tumor tissue indicate that the steroid is rapidly metabolized by O-methylation by the action of catechol O-methyltransferase in the tissue to form a metabolically stable but inactive derivative (Fig. 1). 2-Methoxyestrone is generally regarded as a physiologically inert metabolite of the catechol estrogen although it has been shown to possess a hypcholesteremic activity (25, 26); it is, however, devoid of the uterotropic activity (18), inactive as an effector of gonadotrophin and prolactin secretion (18), and has no affinity for the estrogen receptor (5). It seems therefore that the initial failure to observe the effect of the catechol estrogen on the cell growth and proliferation of the MCF-7 cells can be ascribed to its rapid metabolism by the enzyme. When the tissue catechol O-methyltransferase activity was diminished by the inclusion...
of a catechol O-methyltransferase inhibitor, quinalizarin (19),
the antiestrogenic activity of the catechol estrogen could be
expressed. It has been reported that in human breast cancer
tissues catechol O-methyltransferase was elevated 3- to 20-
fold relative to the normal human mammary gland (23, 27).
In line with these data is our present result indicating that
this enzyme activity is also greatly elevated in the cultured
human breast cancer MCF-7 cells. It seems therefore likely
that catechol O-methyltransferase plays a role in the overall
expression of the "estrogenic" action of estradiol in many if
not all estrogen target tissues where the primary estrogen is
converted to the catechol estrogen or which are accessible to
catechol estrogen produced elsewhere. The recent data by
Hersey et al. (28) which indicates that the inhibition of uterine
catechol O-methyltransferase by an inhibitor, U-0521, poten-
tiates the action of catechol estrogen on the synthesis of
induced protein is another case in point albeit in this instance
the agonist activity of the catechol estrogen was apparently
enhanced. Although catechol O-methyltransferase is known
to be an ubiquitous enzyme its exact cellular and subcellular
localization and control, especially in estrogen target tissues,
has not been determined. Information on its localization and
activity in relation to estrogen 2-hydroxylase could therefore
add to our understanding of the role of the aromatic hydrox-
ylation and the subsequent O-methylation reactions in the
expression of estrogen action. Our present data indicating
that the enzyme plays an important role in the expression of
the catechol estrogen action in an estrogen-responsive cancer
cell line MCF-7 is indicative of a possible role for this enzyme
in the regulation of hormonal actions of estrogens in normal
target tissues.

The actions of 2-hydroxyestrone in the presence of the
catechol O-methyltransferase inhibitor on the MCF-7 cell
cultures are similar to those exerted by the synthetic triphen-
ylethylene estrogen antagonists such as tamoxifen. These
substances also inhibit cellular proliferation in estrogen re-
ceptor-positive cells (21), an action which can be reversed at
an early stage by estradiol but which also becomes irreversible
upon prolonged exposure to these agents (21, 29). It has been
proposed that these antiestrogens act via estrogen receptor-
related nuclear events at the level of chromatin or at the level
of nuclear receptor "processing" (30). The 2-hydroxyestrogens
also mimic the synthetic nonsteroidal antiestrogens in their
in vivo mode of action; they behave as mixed estrogen ago-
nists-antagonists and like the synthetics are capable of acting
as estrogens under certain circumstances and as antiestrogens
under others (2-4).

The irreversible nature of the catechol estrogen inhibition of
MCF-7 cell growth after 4 days of exposure to catechol
estrone (Fig. 4) may shed some light on the mechanism by
which the catechol estrogen exerts its effect on the tumor cell
growth and proliferation. It is evident that the reversible
action of the catechol estrogen is mediated through some
estrogen receptor-related event since the primary estrogen
can reverse the action of the catechol estrogen when present
simultaneously on day 1 (Fig. 3). In the absence of the
estrone, however, it is considered that the catechol estrogen
affects irreversibly a component involved in the estrogen
receptor-mediated event, rendering it unresponsive to the
subsequent stimulation of the estrogen. The 2-hydroxyestro-
gens readily undergo oxidation to form powerful semiquinone-
like electrophiles which can bind covalently to the nucleo-
philic sites of biological macromolecules (31, 32). It is, there-
fore, possible that the irreversible action of 2-hydroxyestrone
on the cell growth of MCF-7 is the result of such covalent
bonding to cellular macromolecules including presumably the
estrogen receptor itself. The same mechanism for the irre-
versibility of action can be invoked in the case of the triphen-
ylethylene estrogen antagonists. These substances are subject
to oxidative metabolism (33) leading to phenolic derivatives
with the potential for similar covalent binding either directly
or via further transformed derivatives. In this context it is
interesting that Reddel et al. (34) have reported the reversal
of the inhibitory action of 4-hydroxytamoxifen on MCF-7 cell
growth requires many fold greater concentrations of estradiol
than in the case of tamoxifen. It is also possible that the
action of the catechol estrogen may also be mediated through
its binding to an antiestrogen binding site reportedly found
in the cytosol of many estrogen-responsive cells (35) including
the MCF-7 human breast cancer cells (36), which is distinct
from the classical estrogen receptor (35, 37). Until the me-
chanism of catechol estrogen on antiestrogen action on MCF-7
cells is more precisely known, it is not possible to define
whether the noted similarities reflect common biological path-
ways or whether the catechol estrogen acts as the endogenous
analogue of the triphenylethylene drugs.

The present data provide evidence that 2-hydroxyestrone,
a natural and qualitatively significant estrogen, can inhibit
the growth of the estrogen-dependent human breast tumor
cell line MCF-7. This is accomplished at a concentration,
which although not physiological, is orders of magnitude lower
than any inhibitory concentration of the other estrogens. As
noted previously the exposure to catechol O-methyltran-
ferase and biological availability of endogenous intracellularly
produced catechol estrogen may differ greatly in a quantita-
tive sense from exogenously supplied 2-hydroxyestrone. The
mechanism of this inhibition is unclear but strong evidence
suggests the involvement of estrogen receptor. What is clear,
however, is that the action of the catechol estrogen is inhibited
by the extraordinarily high activity of catechol O-methyl-
transferase present in these tumor cells. It is tempting to
speculate that this distortion in catechol O-methyltransferase
activity may have been one of the precipitating events which
led to the disruption of cellular control of estrogen expression
and to eventual cell transformation. The clear estrogen
agonist activity of the 16α-hydroxylated metabolites of estradiol
which are unaffected by catechol O-methyltransferase pres-
ence but which regulate its activity emphasize further that
the direction of estradiol metabolism either in situ or in the
periphery can have a profound influence on the expression of
its hormonal activity.

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