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Differences in the Mechanism of the Inhibitory Actions of Catecholestrogens, Tamoxifen and High Concentrations of Estrogens on Prolactin Release by Cultured Rat Pituitary Tumor Cells*

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Abstract—*Chronic administration of the catecholestrogens 2-OH-estrone (2-OH_1) and 2-OH-estradiol (2-OHE_2), of tamoxifen and its metabolites and of high concentrations of estradiol have been previously shown to inhibit the growth of the estrogen/progesterone receptor-positive transplantable prolactin (PRL)-secreting rat putuitary tumor 7315a. The mechanism of action of these inhibitory effects on tumor growth is unknown. In the present study we investigated the direct effects of these compounds on PRL release by a tumor cell clone derived from the 7315a tumor.*

E_2 stimulated PRL release in FCS_{ABS} (10% estrogen-stripped fetal calf serum)-cultured tumor cells in a biphasic manner: at low concentrations (0.1–100 nM) there was a dose-dependent stimulation of PRL release, which decreased in response to 1 μM E_2 and which was greatly inhibited by 10 μM E_2. Both 2-OHE_2 (100 nM and 1 μM) and 2-OHE_2 (1 μM) inhibited PRL release by FCS-cultured tumor cells. In FCS_{ABS}-cultured tumor cells, 0.1–10 nM 2-OHE_1 and 1 μM 2-OHE_2 inhibited PRL release, but 1–100 nM 2-OHE_2 stimulated PRL release. Tamoxifen (TMX) and its metabolites dihydroxy (di-OH-TMX) and 4-hydroxy-tamoxifen (4-OH-TMX) inhibited PRL in a dose-dependent manner. The PRL release inhibiting effect of 4-OH-TMX was 100 times more potent that those of TMX and di-OH-TMX, which were similar in their effect. The inhibitory effects of micromolar concentrations of the cathecholestrogens on PRL release could be overcome by estradiol, while the inhibitory effects of high concentrations of tamoxifen were not prevented by estradiol.

Both "endogenous" (catecholestrogens) and "exogenous" (tamoxifen and its metabolites) anti-estrogens and very high concentrations of estradiol directly inhibit PRL secretion by cultured pituitary tumor cells. The mechanism of their anti-tumor effects, however, seems to differ. The catecholestrogens have direct anti-estrogenic effects on cultured tumor cells, which can be antagonized by estradiol. The final effect of their mixed antagonistic/agonistic action depends on the presence or absence of estrogens in the culture medium. Tamoxifen also affects tumor growth probably mainly via a direct effect, partly involving anti-estrogenic and partly direct toxic effects.

INTRODUCTION

CONFLICTING data have been reported on the direct effects of estrogens and the interrelationships between estrogens and "endogenous" (catechol-estrogens) and "exogenous" (tamoxifen and its metabolites) anti-estrogens on human mammary and pituitary tumor cell lines: in some studies direct effects of estrogens on tumor cell activity were observed [1, 2], while others failed to show these direct effects [3–5].

We showed before in the model of the estrogen/progesterone receptor-positive transplantable prolactin (PRL)/adrenocorticotropin (ACTH)-secreting rat pituitary tumor 7315a that *in vivo* administration of high doses of estradiol, tamoxifen and of the catecholestrogens 2-hydroxyestrone (2-OHE_1) and 2-hydroxyestradiol (2-OHE_2) inhibited the growth of the pituitary tumor [6, 7]. In order to investigate further the mechanism of these anti-

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tumor effects, we prepared a tumor cell line from the 7315a tumor which secreted only PRL. We investigated the effect of estradiol and the interrelationships between estradiol and the compounds mentioned above on PRL release by these cultured pituitary tumor cells.

MATERIALS AND METHODS

Establishment of a tumor cell line

Female buffalo rats (150–170 g) were inoculated s.c. between the scapulae with homogenates of the PRL/ACTH-secreting pituitary tumor 7315a as described before [8].

Primary cultures of tumor cells were prepared by mincing pieces of tumor in Hank's balanced salt solution (HBSS) with 1% bovine serum albumin, and resuspending the spontaneously dissociated tumor cells in Eagle's minimal essential medium with Earle's salts (MEM) containing 10% fetal calf serum (FCS; Gibco Biocult, Glasgow, U.K.), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 40 μg/ml fungizone, 1% MEM non-essential amino acids (Gibco) and 1 mM sodium pyruvate. The cells were plated at approx. 10^5 cells per 100 mm culture dishes. After 1–2 weeks in culture and regular medium changes (once every 3 or 4 days), the surviving tumor cells were harvested using trypsin and resuspended in fresh culture medium. After 10 serial passages in culture, single cell plating techniques resulted in several purely PRL-secreting clonal tumor cell lines. One of these was recloned many times and the present studies were carried out with this cell line.

Tumor cell experiments

One hundred thousand (10^5) tumor cells were allowed to attach to Falcon Petri dishes in 1 ml culture medium at 37°C in humidified air with 5% CO_2. The culture medium consisted of the medium mentioned above supplemented with 10% FCS or 10% estrogen-stripped FCS (FCS_{ABS}; see below). Media and drugs investigated were changed every 3rd or 4th day, and the experiments were carried out for 7–18 days.

Tamoxifen, 4-hydroxytamoxifen (4-OH-TMX), dihydroxytamoxifen (di-OH-TMX) were a generous gift of Dr. B. Furr (ICI, Macclesfield, U.K.). The 2-hydroxylated derivatives 2,3-dihydroxy-1,3,5-(10)-estratrien-17-one (2-OHE_1), 1,3,5-(10)-estratrien-2,3-17β-triol (2-OHE_2) and 17β-estradiol were obtained as 99% pure substances from Sigma (St. Louis, MO, U.S.A.). All compounds were solubilized in absolute ethanol and further diluted in culture medium. The final ethanol concentration was less than 0.1% and this percentage was also added to control dishes not containing these drugs. At the end of an experiment the media were removed and centrifuged at 1000 ***g*** and the supernatants stored at - 20°C. In some instances the PRL content and protein content of the attached tumor cells were investigated. In those cases the cells were scrubbed with a rubber policeman and exposed to repeated freezing and thawing, before the PRL and protein content were measured.

In some experiments charcoal-treated estrogen-stripped fetal calf serum was used. The estradiol-17β content of the original calf serum used in these studies amounted to 80–100 pmol/l (final concentration in the dishes about 10 pmol/dish), while 17β-E_2 concentrations had become undetectable after treatment for 30 min at room temperature with 0.5% (w/v) Norit and 0.05% (w/v) dextran T-70. Charcoal was removed by centrifugation for 30 min at 10,000 ***g***. The supernatant is referred to as dextran-coated charcoal treated FCS = FCS_{ABS}.

Rat PRL assay and statistics

Rat PRL concentration of the culture media and the tumor cells was measured by double antibody RIA procedures using materials and protocols supplied by the hormone distribution officer of the NIADDK. The protein content of the tumor cell-solution was estimated using a reagent kit from Bio-Rad (Richmond, CA, U.S.A.) with bovine albumin (Kabi, Stockholm, Sweden) as a standard.

All samples were assayed in duplicate. Statistical analysis was done by analysis of variance, followed by Duncan's test for determining difference between control and experimental groups.

RESULTS

PRL release by the cultured pituitary tumor cells was followed for 14 days (Table 1). PRL release/24 hr rapidly increased with time. Hormone release by the tumor cells cultured in FCS_{ABS} released significantly less PRL at all time points ($P < 0.01$ vs. FCS-cultured tumor cells after 3, 7, 10 and 14 days of culture). Estradiol (1 nM) did not influence PRL release by tumor cells cultured in 10% FCS, but significantly stimulated PRL release of tumor cells cultured in 10% FCS_{ABS} ($P < 0.01$ vs. FCS_{ABS}-cultured control cells after 7, 10 and 14 days of culture). Tamoxifen (1 μM) significantly inhibited PRL release by cells cultured in FCS ($P < 0.01$ vs. control), while 100 nM and 1 μM tamoxifen inhibited PRL release by tumor cells cultured in FCS_{ABS} to an even higher extent ($P < 0.01$ vs. control FCS_{ABS}-cultured cells for both concentrations of tamoxifen). The percentage decrease of PRL release by 100 nM and 1 μM tamoxifen in FCS_{ABS} was significantly higher ($P< 0.01$ and $P < 0.01$, respectively), than that exerted by 1 μM tamoxifen in FCS-cultured tumor cells. The inhibitory effect of 100 nM tamoxifen on PRL release by tumor cells cultured in 10% FCS_{ABS}

Table 1. The influence of dextran-charcoal treatment of FCS on basal PRL release and on the effects of estradiol (E_2) and tamoxifen on PRL release by cultured tumor cells prepared from the 7315a tumor

	PRL release (ng/dish/24 hr)			
	Day 3	Day 7	Day 10	Day 14
Cells cultured in FCS				
Control	55 ± 3	526 ± 30	2593 ± 126	6401 ± 360
E_2 (1 nM)	50 ± 4	519 ± 32	2245 ± 86	6016 ± 420
Tamoxifen (1 μM)	50 ± 3	406 ± 21*	1042 ± 41*	3531 ± 201*
Cells cultured in FCS_{ABS}				
Control	26 ± 2†	145 ± 11†	420 ± 21†	1345 ± 86†
E_2 (1 nM)	35 ± 3‡	205 ± 8‡	490 ± 16‡	1778 ± 72‡
Tamoxifen (100 nM)	25 ± 3	86 ± 6‡	135 ± 11‡	505 ± 31‡
Tamoxifen (1 μM)	21 ± 2	48 ± 6‡§	43 ± 7‡§	133 ± 12‡§
E_2 (1 nM) + tamoxifen (100 nM)	25 ± 2	102 ± 7‖	268 ± 12‖	872 ± 38‖

Mean ± S.E.M.; $n = 5$ dishes per group.
*$P < 0.01$ vs. control.
†$P < 0.01$ vs. FCS-control.
‡$P < 0.01$ vs. FCS_{ABS}-control.
§$P < 0.01$ vs. tamoxifen 100 nM in FCS_{ABS}.
‖$P < 0.01$ vs. tamoxifen 100 nM.

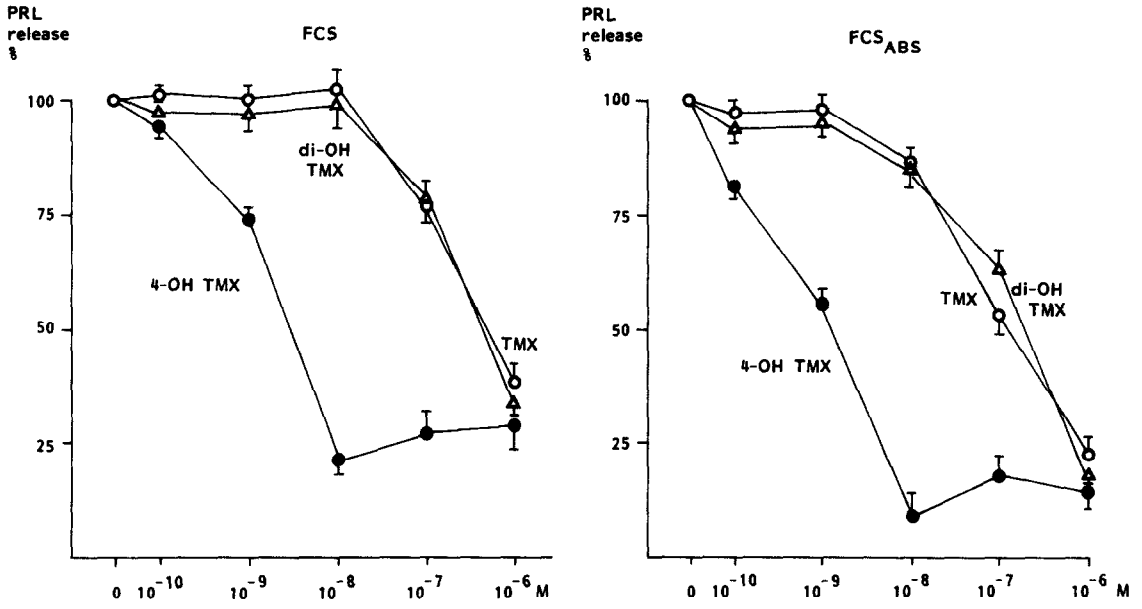

Fig. 1. The effects of tamoxifen (TMX), dihyroxytamoxifen (di-OH-TMX) and 4-hydroxytamoxifen (4-OH-TMX) on PRL release by FCS (left) or FCS_{ABS} (right) cultured 7315a tumor cells. PRL release for 10 days is expressed as a percentage of control. Mean ± S.E.M.; n = 4 dishes per group.

could be significantly overcome by 1 nM estradiol (E_2) ($P < 0.01$ vs. tamoxifen alone).

Dose–response studies of tamoxifen (TMX) and its metabolites dihydroxytamoxifen (di-OH-TMX) and 4-hydroxytamoxifen (4-OH-TMX) on PRL release by the 7315a tumor cell clone are shown in Fig. 1. TMX and di-OH-TMX (100 nM) significantly inhibited PRL release by tumor cells cultured for 7 days in 10% FCS by 24 and 22%, respectively ($P < 0.01$ vs. control in both instances), while 1 μM of both compounds inhibited PRL release by more than 50%. 4-OH-TMX was at least 100 times more potent in its PRL-release inhibitory action (Fig. 1, left): 1 nM 4-OH-TMX inhibited PRL release by 25% ($P < 0.01$ vs. control), while 10 nM, 100 nM and 1 μM of the compound inhibited hormone release by about 70%. The inhibitory effects of these three compounds on PRL release by tumor cells cultured in FCS_{ABS} were significantly more powerful than those observed in FCS-cultured cells. TMX and di-OH-TMX (10 nM) already significantly inhibited PRL release by 14 ± 3 and 15 ± 3%, respectively ($P < 0.05$ vs. control in both instances), while 0.1 nM 4-OH-TMX already inhibited PRL release by 19± 2% ($P < 0.01$ vs. control). The dose–response effects

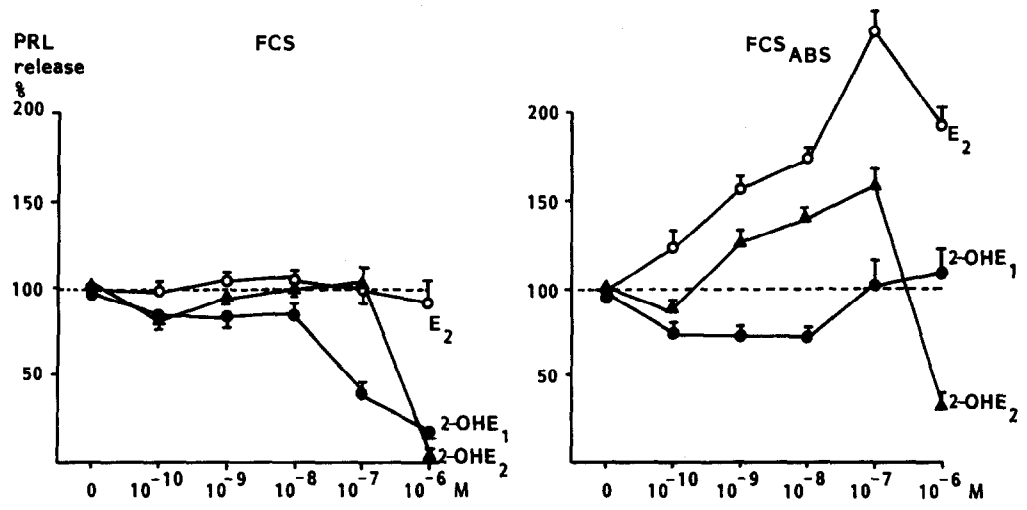

Fig. 2. The effects of estradiol (E_2), 2-hydroxyestrone (2-OHE_1) and 2-hydroxyestradiol (2-OHE_2) on PRL release by FCS (left) or FCS_{ABS} (right) cultured 7315a tumor cells. PRL release for 10 days is expressed as a percentage of control. Mean ± S.E.M.; n = 4 dishes per group.

of the three compounds on PRL release by FCS_{ABS}-cultured cells were similar to those observed in FCS-cultured tumor cells. Only the sensitivity of the PRL-release-inhibiting effects of the three compounds was higher in FCS_{ABS}-cultured cells at concentrations between 0.1 and 10 nM. E_2 (100 nM) completely overcame the inhibitory effect of 100 nM TMX, 4-OH-TMX and di-OH-TMX on PRL release by FCS_{ABS}-cultured tumor cells, but it did not antagonize the effects of 1 μM of these compounds, suggesting a cytotoxic action of this concentration of these drugs.

The actions of the catecholestrogens 2-OHE_1 and 2-OHE_2 on PRL release were compared with the effects of E_2 (Fig. 2). In tumor cells cultured in FCS 100 nM and 1 μM 2-OHE_1 inhibited PRL release significantly in a dose-dependent manner by 55 ± 5 and 83 ± 2%, respectively ($P < 0.01$ vs. control in both instances and $P < 0.01$ between 100 nM and 1 μM 2-OHE_1). Only 1 μM 2-OHE_2 inhibited PRL release significantly (-93 ± 3%; $P < 0.01$ vs. control), while E_2 did not affect PRL release by FCS-cultured tumor cells over a concentration range of from 0.1 nM to 1 μM. If the tumor cells were cultured in FCS_{ABS}, however, a different pattern of effects of these three hormones was observed on PRL release. E_2 stimulated PRL release in a dose-dependent manner (the effect of 100 nM E_2 being significantly higher than that of 0.1, 1 and 10 nM E_2; $P < 0.01$). Interestingly 1 μM E_2 stimulated PRL release significantly less than 100 nM of this hormone ($P < 0.01$). In a separate experiment it was shown that 10 μM E_2 inhibited PRL release by 70 ± 5% ($P < 0.01$; data not shown). 2-OHE_1 at low concentrations of 0.1, 1 and 10 nM, inhibited PRL release by 26 ± 2, 27 ± 3 and 28 ± 4%, respectively ($P < 0.01$ vs. control in all instances), while 100 nM and 1 μM of this compound were without effect. 2-OHE_2 also affected PRL release in a biphasic manner: 1, 10 and 100 nM 2-OHE_2 stimulated PRL release in a dose-dependent manner ($P < 0.01$ for 100 nM vs. 1 nM 2-OHE_2), while 1 μM 2-OHE_2 inhibited PRL release by 70 ± 3% ($P < 0.01$ vs. control). The data presented in Fig. 2 show the results of a representative experiment, which was repeated three times with similar results. The inhibitory effect of 1 μM 2-OHE_1 and 2-OHE_2 on PRL release by tumor cells cultured in FCS_{ABS} could be completely overcome by co-incubation with 100 nM E_2.

In this study the effect of different (anti)-estrogens was shown on PRL release by a pituitary tumor cell clone. Independent of the estrogen content of the culture medium, the number of plated cells and the duration of incubation, there was a close correlation between the amount of PRL released into the medium and the PRL content of the tumor cells ($P < 0.001$). About nine times more PRL was found in the medium than intracellularly in all instances. In addition there was a close correlation between PRL release into the medium, the PRL content of the tumor cells and the protein and DNA content of the tumor cells ($P < 0.001$ in all instances).

DISCUSSION

Many of the studies on the mechanism of action of tamoxifen, its metabolites and catecholestrogens have been carried out in the estrogen-receptor positive human breast cancer cell line MCF-7 or other similar cell lines [9–11]. PRL-secreting normal or tumorous rat pituitary cells are also good models to study the effects of endogenous and/or exogenous (anti)-estrogens, as changes in (tumor) cell activity are in general well mirrored by changes in the rate of synthesis and release of PRL [12].

Earlier studies showed a clear stimulation by estrogens of cell growth and PRL synthesis and release in the GH_4C_1 cell line if it was cultured in castrated horse serum [13], and in cell lines prepared from the MtT/W13 tumor [12], while (monohydroxy)-tamoxifen strongly inhibited the synthesis of PRL by GH_3 rat pituitary tumor cells [14]. We reported in the present study only the effects of different endogenous and exogenous (anti)-estrogens on PRL release by the cultured pituitary tumor cell clone. There was in all instances a close correlation, however, between the PRL concentration of the incubation medium, the PRL content of the tumor cells and the protein and DNA content of these cells. This observation suggests that PRL release by these tumor cells also represents a measure of the mitotic activity of this cell line, and that the stimulating and inhibitory effects on PRL release of the compounds used in this study also reflect stimulation and inhibition of tumor cell growth.

In the present study we used a tumor cell line prepared from the PRL/ACTH-secreting rat pituitary tumor 7315a which contains 534 fmol estrogen receptor/mg protein and 70 fmol progesterone receptor/mg protein (determinations kindly carried out by Dr. M.A. Blankenstein). This tumor cell line secreted only PRL. PRL release by the cultured 7315a tumor cells was greatly dependent on whether the cells were cultured in FCS or FCS_{ABS}. In the absence of estrogens in the culture medium (FCS_{ABS}) E_2 stimulated PRL release in a dose-dependent manner. This observation further underlines the observations by Lippman [2] in MCF-7 cells and of Amara and Dannies [13] in pituitary cell lines that studies on the direct effects of estrogens on cell growth or hormonal activity of cultured cell lines should be carried out utilizing estrogen-deficient culture media. The observation that 1 μM E_2 exerted a less powerful stimulation of PRL release than 10 nM of the hormone, while 10 μM E_2 strongly inhibited PRL release was in line with earlier *in vivo* observations. We showed before that chronic administration of a high dose estradiol (10 μg/kg/day) virtually completely prevented the growth of the estrogen-receptor positive 7315a tumor [7], while similar observations have been reported with regard to the growth of rat mammary tumors [15]. The present study suggests that very high concentrations of E_2 inhibit tumor growth via a direct toxic effect on the tumor and not indirectly via inhibition of PRL stimulation *in vivo* [16].

Non-steroidal anti-estrogens like tamoxifen inhibit many of the actions of E_2 on target tissues. It was shown before that an E_2-stimulated increase in PRL synthesis by primary cultures of rat pituitary cells is competitively inhibited by tamoxifen and 4-OH-tamoxifen [17, 18]. In the present study we compared the effects of tamoxifen and two of its metabolites on PRL release by cultured tumor cells. 4-OH-Tamoxifen is a metabolite of tamoxifen with a potent anti-estrogenic activity with an estrogen receptor binding activity similar to that of E_2 [19]. In the rat 4-OH-tamoxifen is further metabolized to dihydroxytamoxifen, which has similar anti-estrogenic properties as tamoxifen [20]. Both tamoxifen and dihydroxytamoxifen have relative binding activities to the estrogen receptor of only 3% of that of E_2 [19, 20]. Our studies clearly show an inhibition of PRL release by these three compounds which parallels the relative affinity of these drugs to the estrogen receptor: 4-OH-tamoxifen was about 100 times more potent in its PRL-release-inhibitory capacity than (dihydroxy)-tamoxifen.

The catecholestrogens $2\text{-}OHE_1$ and $2\text{-}OHE_2$ are quantitatively the principle metabolites of estradiol [21]. From many studies in tissues such as the pituitary gland, the hypothalamus and cultured human breast cancer cell lines, it has been concluded that after *in situ* formation of the catecholestrogens from estradiol, these compounds might function as endogenous anti-estrogens, subserving a physiological mechanism by which these compounds exert a negative regulatory control to oppose the angonistic actions of the primary estrogen [21–23]. $2\text{-}OHE_1$ and $2\text{-}OHE_2$ have been shown to differ considerably, however, with regard to their effects in several models. $2\text{-}OHE_1$ inhibited PRL release by normal rat anterior pituitary cells cultured in charcoal-treated estrogen-stripped FCS in a biphasic manner [24]: 100 nM and 1 μM $2\text{-}OHE_1$ inhibited normal PRL release by 20 and 38%, respectively, while this effect disappeared at higher concentrations. $2\text{-}OHE_1$ (10 nM and 100 nM) also significantly inhibited the growth and proliferation of MCF-7 human breast cancer cells [11]. This effect of $2\text{-}OHE_1$ was neutralized, however, in the presence of 1 nM E_2. The results presented in this study are in agreement with these observations of a predominantly estrogen antagonistic and at higher concentrations in the absence of E_2 mildly agonistic activity of $2\text{-}OHE_1$.

Dual actions of $2\text{-}OHE_2$ have also been reported. This compound combines estrogen agonistic and antagonistic properties depending on the concentration used [21–23]. In normal PRL-secreting rat pituitary cells cultured in FCS_{ABS} 100 nM $2\text{-}OHE_2$ already maximally inhibited PRL release (−37%), while the effect of 1 μM of the compound on PRL release was significantly less [24]. Similar mixed antagonist/agonist actions of $2\text{-}OHE_2$ have been observed in the uterus [22] and on PRL secretion in ovariectomized [25] and young male rats [26]. The powerful estrogen-agonistic activity of $2\text{-}OHE_2$ at low concentrations was also clear in our experi-

ments with the PRL-secreting 7315a cell clone cultured in estrogen-stripped FCS. The estrogen-antagonistic action of higher concentrations (1 μM) of the compound caused a clear inhibition of PRL release both in FCS and FCS_{ABS}-cultured tumor cells. The mechanisms underlying the action of $2\text{-}OHE_1$ and $2\text{-}OHE_2$ indeed probably involve competition at the estrogen receptor, as both compounds have been shown to bind with the estrogen cytosolic receptors [27, 28] and are subsequently translocated into the nucleus [29]. In addition the effects of both catecholestrogens were antagonized by low concentrations of E_2 [11, 24; this study]. The results presented in this study suggest that the inhibitory action of the chronic *in vivo* administration of $2\text{-}OHE_1$ and $2\text{-}OHE_2$ on the growth of the 7315a tumor seems to be at least partly caused by a direct anti-tumor effect of these compounds. Our studies also suggest a different mechanism of action of the catecholestrogens and tamoxifen. The inhibitory effects of micromolar concentrations of the "endogenous" anti-estrogens in PRL release could be antagonized by E_2, and those by tamoxifen and its derivatives not.

It is surprising that compounds like tamoxifen and its derivatives exert such explicit inhibitory actions on PRL release by pituitary tumor cells cultured in 10% FCS. In other systems, such as cultured human breast cancer cells, inhibition of tumor cell growth was not even seen by 1 μM tamoxifen, if the cells were cultured in 10% dextran charcoal-treated fetal calf serum [1]. This absence of effect was ascribed to tamoxifen binding components in the serum. It cannot be readily explained at present what causes the differences in sensitivity of these two model systems to tamoxifen.

Recently it was shown that phenol red in concentrations used in most tissue culture media acts as a weak estrogen [30]. Our experiments were carried out with media containing phenol red. Therefore it might not be justified to discuss the results of the experiments carried out with 10% FCS_{ABS} as being "in the absence of estrogens". It is at present uncertain whether the presence of phenol red in the culture medium used has affected the effect of PRL release of the compounds used.

In conclusion we showed in the present study that high concentrations of estradiol, "endogenous" (catecholestrogens) and "exogenous" (tamoxifen) anti-estrogens inhibit the growth of an estrogen/progesterone receptor positive transplantable PRL-secreting rat pituitary tumor. The catecholestrogens had direct anti-estrogenic effects on the tumor, but their action was shown to be antagonized by and also highly dependent on the presence and concentration of estrogens. Tamoxifen itself or one or more of its metabolites, however, had powerful direct inhibitory effects on PRL-release and probably also on the growth on the 7315a tumor, which at high concentrations could not be antagonized by E_2.

REFERENCES

1. Darbre P, Yates J, Curtis S, King RJB. Effect of estradiol on human breast cancer cells in culture. *Cancer Res* 1983, **43**, 349–354.
2. Lippman M. Hormonal regulation of human breast cancer cells *in vitro*. *Banbury Rep* 1981, **8**, 171–181.
3. Lee H, Davies IJ, Soto AM, Sonnenschein C. Estrogen induction of progesterone receptor and its relationship to cell multiplication rate in the rat pituitary tumor cell line C_2-9RAP. *Endocrinology* 1981, **108**, 990–996.
4. Sirbuska D. Estrogen induction of growth factors specific for hormone responsive mammary, pituitary, and kidney tumor cells. *Proc Natn Acad Sci USA* 1978, **75**, 3786–3790.
5. Sonnenschein C, Soto AM. But . . . are estrogens *per se* growth-promoting hormones? *J Natn Cancer Inst* 1984, **64**, 211–215.
6. Lamberts SWJ, Uitterlinden P, Zuiderwijk-Roest JM, Bons-van Evelingen EG, de Jong FH. Effects of a luteinizing hormone-releasing hormone analog and tamoxifen on the growth of an estrogen-induced prolactin/secreting rat pituitary tumor and its influence on pituitary gonadotropins. *Endocrinology* 1981, **108**, 1878–1884.
7. Lamberts SWJ, Nagy I, Uitterlinden P, MacLeod RM. The effect of catecholestrogens on the growth of prolactin-secreting pituitary tumors and normal prolactin synthesis in the rat. *Endocrinology* 1982, **110**, 1141–1146.
8. Lamberts SWJ, Uitterlinden P, Bons EG, Verleun T. Comparison of the actions of RU 38486 and megestrol acetate in the model of a transplantable adrenocorticotropin- and prolactin-secreting rat pituitary tumor. *Cancer Res* 1985, **45**, 1015–1021.
9. Horwitz KB, Koseki Y, McGuire WL. Estrogen control of progesterone receptor in human breast cancer. Role of estradiol and antiestrogen. *Endocrinology* 1978, **103**, 1742–1751.
10. Lipman M, Bolan G, Huff K. The effect of estrogens and antiestrogens on hormone responsive human breast cancer in long-term tissue culture. *Cancer Res* 1976, **36**, 4595–4601.
11. Schneider J, Huh MM, Bradlow L, Pishman J. Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. *J Biol Chem* 1984, **259**, 4840–4845.
12. Posner M, Rosner AL, Pellegrini J, Burstein NA. Establishment of estrogen-responsive clonal cell lines in tissue culture from an estrogen-dependent, prolactin-secreting rat

pituitary tumor. *Cancer Res Ther* 1984, **31**, 137–146.
13. Amara JF, Dannies P. 17β-Estradiol has a biphasic effect on GH cell growth. *Endocrinology* 1983, **112**, 1141–1146.
14. Tate AC, Lieberman ME, Jordan VC. The inhibition of prolactin synthesis in GH_3 rat pituitary tumor cells by monohydroxytamoxifen is associated with changes in the properties of the estrogen receptor. *J Steroid Biochem* 1984, **20**, 391–395.
15. Lemon HM. Estriol prevention of mammary carcinoma induced by 7,12-dimethylbenzanthracene and procarbazine. *Cancer Res* 1975, **35**, 1341–1346.
16. Meites J, Cassell E, Clard J. Estrogen inhibition of mammary tumor growth in rats: concentration by prolactin. *Proc Soc Exp Biol Med* 171, **137**, 1225–1229.
17. Lieberman MA, Graig-Jordan V, Fritsch M, Amparo Santos M, Gorski J. Direct and reversible inhibition of estradiol-stimulated prolactin synthesis by antiestrogens *in vitro*. *J Biol Chem* 1983, **258**, 4738–4740.
18. Lieberman MA, Gorski J, Graig-Jordan V. An estrogen receptor model to describe the regulation of prolactin synthesis by antiestrogens *in vitro*. *J Biol Chem* 1983, **258**, 4741–4745.
19. Skidmore JR, Walpole AL, Woodburn J. Effect of some triphenylethylenes on estradiol binding *in vitro* to macromolecules from uterus and anterior putuitary. *J Endocrinol* 172, **52**, 289–298.
20. Fromson JM, Pearson S, Bramah S. The metabolism of tamoxifen (ICI 46, 474): Part I in laboratory animals. *Xenobiotica* 1973, **3**, 693–709.
21. Fishman J. Role of 2-hydroxyestrone in estrogen metabolism. *J Clin Endocrinol Metab* 1963, **23**, 207–218.
22. Fishman J. Biological action of catechol oestrogens. *J Endocrinol* 1981, **89**, 59–72.
23. Paul SM, Hoffman AR, Axelrod J. Catechol estrogens: synthesis and metabolism in brain and other endocrine tissues. In: Ganong WR, Martini L, eds. *Frontiers in Neuroendocrinology*. New York, Raven Press, 1980, 203–217.
24. Lamberts SWJ, Verleun T, Oosterom R. Studies on the effects of catecholestrogens on prolactin secretion by cultured normal rat anterior pituitary cells. *Endocrinology* 1985, **116**, 2631–2636.
25. Yanai R, Nagasawa H. Oestrogenic effecs of catechol oestrogens on the secretion of prolactin by the pituitary gland of synthesis of DNA by the mammary gland in ovariectomized rats. *J Endocrinol* 1979, **82**, 131–133.
26. Barbieri RL, Todd RB, Morishita H, Ryan K, Fishman J, Naftolin F. Response of serum prolactin to catchol estrogen in the immature rat. *Fertil Steril* 1980, **34**, 391–394.
27. Davies IJ, Naftolin F, Ryan KJ, Fishman J, Sin J. The affinity of catechol estrogens for estrogen receptors in the pituitary and anterior hypothalamus of the rat. *Endocrinology* 1975, **97**, 554–557.
28. Martucci C, Fishman J. Uterine estrogen rceptor binding of catecholestrogens and of estradiol (1,3,4(10)-estratriene-3,15,16,17-tetrol). *Steroids* 1976, **37**, 325–333.
29. Weinberger MJ, Aten RF, Eisenfeld AJ. Estrogen receptor in the mammalian liver: effect of metabolism on the amount and identity of receptor-bound estrogen. *Biochem Pharmacol* 1986, **27**, 2469–2474.
30. Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc Natn Acad Sci USA* 1986, **83**, 2496–2500.