Fatty acid modulation of antiestrogen action and antiestrogen-binding protein in cultured lymphoid cells

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Summary. Nonsteroidal antiestrogens reversibly and specifically inhibited the proliferation of two estrogen receptor-negative lymphoid cell lines (EL4 and Raji) in a dose-dependent manner. [^{3}H]Thymidine incorporation of concanavalin A-stimulated primary splenocytes was also inhibited by 10^{-6} M clomiphene (1-[4-(2-diethylaminoethoxy)phenyl]-1,2-diphenyl-2-chloroethylene). The antiproliferative effect could be prevented by the simultaneous presence in the growth medium of 10^{-5} M linoleic acid or 10^{-5} M arachidonic acid but not by 10^{-6} M estradiol. Both lymphoid cell lines had high affinity antiestrogen-binding sites whose affinity could be altered by conditions of growth. Growth of EL4 cells in RPMI 1640 medium supplemented with charcoal-pretreated 5% fetal calf serum (charcoal-stripped medium) resulted in significantly higher affinity (K_d 0.54 nM $\pm$ 0.11 nM; n = 6) than growth in medium supplemented with untreated serum (complete medium) (K_d = 1.68 nM $\pm$ 0.48 nM; n = 6) ($p < 0.001$). This change in affinity was partly due to removal of fatty acids from the growth medium by charcoal pretreatment, since addition of 10^{-5} M linoleic acid or 10^{-5} M gamma-linolenic to charcoal-stripped medium decreased the affinity of the antiestrogen-binding protein. In contrast, growth in 10^{-5} M stearic acid or 10^{-5} M oleic acid did not significantly alter the affinity of the antiestrogen-binding protein, whereas 10^{-5} M palmitic acid significantly increased its affinity. The same fatty acids were also tested for their intrinsic effects on EL4 cell proliferation. Oleic, linoleic and gamma-linolenic acids were growth stimulatory while stearic and palmitic acids were not. Thus linoleic and gamma-linolenic acids whose presence in the growth medium was associated with decreased affinity of [^{3}H]tamoxifen (1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1(Z)-ene) binding to the intracellular antiestrogen-binding protein were also growth stimulatory. Unsaturated fatty acids have previously been shown to inhibit binding of [^{3}H]tamoxifen to the antiestrogen-binding protein in a cell-free system. The present observations demonstrate that unsaturated fatty acids also modify the affinity of the antiestrogen-binding protein in intact cells.
Key words. Antiestrogens; antiestrogen-binding protein; cell proliferation; fatty acids; lymphocytes; estrogen receptor.

That antiestrogens inhibit the proliferation of estrogen target cells is well known. There appear to be at least two mechanisms by which antiestrogens restrain cell division. At nanomolar concentrations, the inhibition is reversible by physiological concentrations of estradiol, which suggests that this action is mediated by the estrogen receptor. This is further supported by the excellent correlation of the growth inhibitory potency of antiestrogens with their affinity of binding to the estrogen receptor[1]. Micromolar concentrations of antiestrogens, however, are cytostatic by mechanism(s) that are probably unrelated to estrogen antagonism and independent of interaction with the estrogen receptor, because the inhibition of cell proliferation under these conditions is not reversible by estrogen and shows no relationship with binding affinity for the estrogen receptor in breast cancer-derived cell lines[2]. This receptor-independent cytostatic effect, nonetheless, has features of specificity. It has, firstly, been observed only for this group of compounds, with an order of potency for this action different from that observed for estrogen receptor-mediated growth inhibition. Secondly, the effect appears to occur in a specific part of the cell cycle, namely in mid-G_1[3]. Possible non-receptor mediated mechanisms of antiestrogen action include inhibition of protein kinase C[4], alterations in transmembrane calcium fluxes[5], calmodulin antagonism[6], and interaction with the antiestrogen-binding protein[7]. The latter is a ubiquitous, intracellular protein of higher eukaryotes which binds nonsteroidal antiestrogens with high affinity and specificity[8]. The antiestrogen-binding protein is located principally in the membranes of the endoplasmic reticulum[9,10] although specific [^{3}H]tamoxifen-binding sites have also been identified in the nucleus and cytosol[8,11,12]. The function of the antiestrogen-binding protein is unknown. In particular, it is unclear whether it is involved at all in estrogen antagonism by this class of drugs in estrogen target cells, although the balance of observations at present suggests that it is not.

If the mechanism of the cytostatic effect of micromolar concentrations of antiestrogens on estrogen target cells is truly independent of the estrogen receptor, we reasoned that the effect should also occur in cell types that lack the receptor and which are not usually considered to be targets of estrogen action. We therefore examined transformed as well as primary lymphocytes for the ability to be growth-inhibited by antiestrogens in a specific and reversible manner. Our findings demonstrate that antiestrogens are antiproliferative in estrogen-insensitive lymphoid cells and that the cytostatic effect can be reversed by unsaturated fatty acids, though not by estradiol. Furthermore, the affinity of the antiestrogen-binding protein in these lymphoid cells is modulated by the presence of fatty acids in the growth medium in a manner

which parallels their ability to inhibit lymphoid cell proliferation.

Materials and methods

Chemicals: [N-methyl-^{3}H]Tamoxifen (3.04 TBq/mmol) and [methyl-^{3}H]thymidine (185 GBq/mmol) were purchased from Amersham International and [2,4,6,7-^{3}H(N)]estradiol (3.34 TBq/mmol) from New England Nuclear. Tamoxifen citrate, clomiphene citrate, fatty acids, concanavalin A, mycoplasma-free fetal bovine serum and Hepes-buffered RPMI 1640 medium were from Sigma Chemical Company. Nafoxidine (1-{2-[4-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-ethyl}pyrrolidine hydrochloride) and LY 117018 (6-hydroxy-2-(4-hydroxyphenyl)-benzo(b)thien-3-yl p(2-(1-pyrrolidinyl)ethoxyphenyl methanone) were gifts from Upjohn Company and Eli Lilly Inc., respectively.

Cell lines: EL4 cells (of mouse T-lymphoid origin) and Raji cells (of human B-lymphoid origin) were generously provided by Dr K. M. Hui and Prof. S. H. Chan, respectively, National University of Singapore.

Cell culture: EL4 and Raji cells were cultured in Hepes-buffered RPMI 1640 medium containing 1.76 g sodium bicarbonate/L, pH 7.1, supplemented with 5% and 10% fetal bovine serum, respectively (called complete medium). Cells were propagated in 75-cm^2 flasks (Costar, USA) at an initial seeding density of approximately 400,000 cells/ml and subcultured every third day. Twenty-four hours prior to use in growth experiments, cells were transferred to RPMI 1640 medium supplemented with charcoal-pretreated serum[13] (called charcoal-stripped medium). Cell viability was estimated by trypan blue exclusion. Cells were not used for growth experiments unless the percentage of dye-excluding cells was 90% or higher.

Primary splenocytes, free of erythrocytes, were prepared from male C57BL/6 mice[14] and maintained in primary culture for 48 h in charcoal-stripped medium.

Growth experiments: *EL4 and Raji cells lines*. Test compounds dissolved in dimethylsulfoxide were added to cells grown in charcoal-stripped medium and dispensed into triplicate 25-cm^2 flasks in such a way that the final concentration of dimethylsulfoxide was 0.01% (v/v). In experiments involving the use of unsaturated fatty acids, 10^{-5} M alpha-tocopherol was also added to reduce lipid peroxidation[15]. Control flasks received identical concentrations of dimethylsulfoxide and, where applicable, 10^{-5} M alpha-tocopherol. The effect of test compounds on the proliferation of EL4 and Raji cells was monitored by daily cell counts using an electronic particle counter (industrial D model, Coulter Elctronics Ltd., England) standardized weekly against Coulter 4C standards for lymphocytes. The mean initial cell density of each group of triplicate flasks (before addition of test agents) served as the control for that group. Subsequent increases in cell density were calculated as the percentage increase over this control value.

Primary splenocytes. Splenocytes were dispensed in 0.2-ml aliquots into a 96-well plate at a density of 10^6 cells/ml. Three groups of triplicate wells were set up as follows: (i) control unstimulated splenocytes (0.1% (v/v) dimethylsulfoxide, 0.09% (w/v) sodium chloride); (ii) mitogenized splenocytes (concanavalin A dissolved in 0.9% (w/v) sodium chloride to a final concentration of 5 µg/ml); and (iii) splenocytes treated with concanavalin A (5 µg/ml) plus 10^{-6} M clomiphene. Splenocyte viability was verified at the start of the experiment and after 48 h of the above treatments. Proliferation of control and treated primary splenocytes was determined after 48 h in culture by pulsing each well with [^{3}H]thymidine (277.5 kBq/well) for 6 h. The amount of [^{3}H]thymidine incorporated was determined by trapping splenocytes on glass microfiber filters (Whatman GF/F) followed by successive washes of water, 0.9% (w/v) sodium chloride, 5% (w/v) trichloroacetic acid and absolute ethanol. The volume of each wash was 3 ml. The dried filters were counted in 2 ml scintillation cocktail (6 g 2,5-diphenyloxazole, 0.07 g 1,4-*bis*[4-methyl-5-phenyl-2-oxazoly]benzene per liter toluene) in a liquid scintillation spectrometer with a counting efficiency of 50% for tritium.

Assay for antiestrogen-binding sites. EL4 and Raji cells were harvested by centrifugation and washed thrice with 50 ml cold 10 mM Tris-HCl, 1.5 mM EDTA, 10% (v/v) glycerol, pH 7.5, to eliminate possible interference with ligand binding by components of the growth medium. Cell pellets were used fresh or stored at − 70 °C until analysis. All subsequent steps were performed at 4 °C. Washed cell pellets were resuspended in the wash solution (4.8×10^7 EL4 cells/ml; 8×10^6 Raji cells/ml) and disrupted in a Potter-Elvehjem homogenizer (clearance 0.1 mm, 12 strokes). Homogenates were centrifuged at $1200 \times g$ for 15 min (EL4 cells) or $12{,}000 \times g$ for 30 min (Raji cells). [^{3}H]Tamoxifen-binding capacity of the supernatants was analyzed by multiple point saturation analysis as previously described[12].

Assay for estrogen receptor sites. Washed EL4 and Raji cell pellets were homogenized as above in 10 mM Tris-HCl, 1.5 mM EDTA, 20 mM sodium molybdate, 10% (v/v) glycerol, 12 mM monothioglycerol, pH 7.5, centrifuged at $1000 \times g$ and 4 °C for 30 min to obtain the crude nuclear pellet. The supernatant was centrifuged again at $100{,}000 \times g$ and 4 °C for 60 min to obtain the cytosol. Estrogen receptor sites were assayed in both fractions as previously described[16,17].

Results and discussion

Both EL4 and Raji cells had high affinity antiestrogen-binding sites as shown by Scatchard analysis (fig. 1) but no detectable estrogen receptor sites (data not shown). These cell lines were thus suitable for determining whether antiestrogens have biological effects which do not rely on the presence of the estrogen receptor. We found that proliferation of lymphoid cells was inhibited

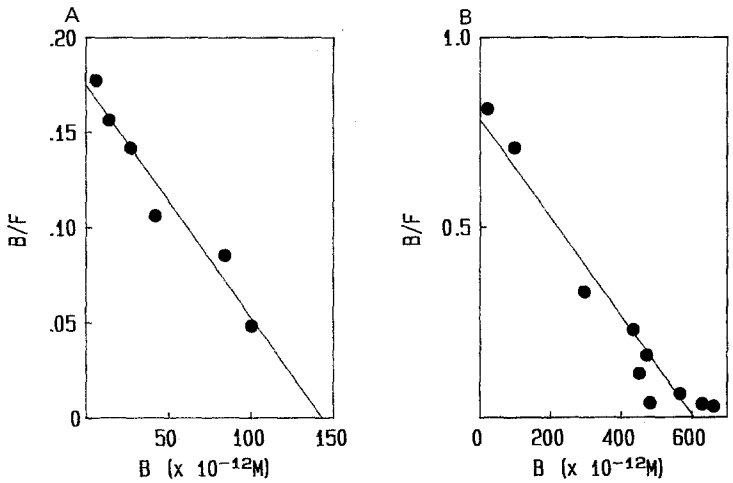

Figure 1. Scatchard plots of [^{3}H]tamoxifen-binding sites in (*A*) EL4 cells and in (*B*) Raji cells.

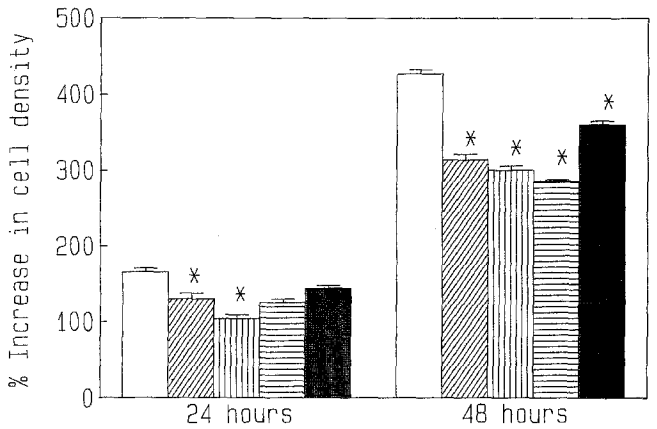

Figure 2. Inhibition of EL4 cell proliferation by nonsteroidal antiestrogens. EL4 cells in charcoal-stripped RPMI 1640 medium were inoculated into triplicate 25-cm^2 flasks at an initial density of about 300,000 cells/ml. Daily cell counts were performed using a Coulter counter. Cell proliferation is expressed as the percentage increase in cell density over the mean initial density of each group of triplicate flasks. Data shown are mean values (columns) and standard deviations (bars). Asterisks denote significant differences from control ($p < 0.05$). All flasks received 0.1% (v/v) dimethylsulfoxide. The following compounds were tested at 10^{-6} M: clomiphene (diagonal lines), nafoxidine (vertical lines), LY 117018 (horizontal lines) and tamoxifen (stippled). Open bars represent flasks treated with 0.1% (v/v) dimethylsulfoxide alone or with 10^{-6} M estradiol, testosterone, dexamethasone, progesterone or quercetin.

by antiestrogens. Figure 2 shows the results of a representative experiment demonstrating that, when seeded at comparable initial densities, EL4 cells cultured in the presence of 10^{-6} M tamoxifen, clomiphene, nafoxidine or LY 117018 increased in cell density more slowly than control cells cultured in the presence of 0.1% (v/v) dimethylsulfoxide alone. Equimolar concentrations of estradiol, testosterone, progesterone, dexamethasone, progesterone and quercetin had no significant effect on EL4 cell proliferation. Growth inhibition by 10^{-6} M clomiphene and 10^{-6} M nafoxidine was evident by 24 h and both continued to exert significant inhibition on cell proliferation after 48 h. The cytostatic effects of tamoxifen and LY 117018 were delayed by comparison, being significant only at 48 h (fig. 2). Thus, among the antiestrogens tested (which were representative of three different structural types), clomiphene and nafoxidine had the most potent growth inhibitory effect. The lower proliferation rates resulting from antiestrogen treatment were not associated with differences in cell viability since this was uniformly high (> 95%) in all groups at the beginning and end of each period of observation.

The antiproliferative effect of antiestrogens on these transformed lymphoid cells was dose-dependent. The degree of growth inhibition was related to clomiphene concentrations between 10^{-8} M and 10^{-6} M, whether growth rate was assessed by increase in cell density (fig. 3) or by [^{3}H]thymidine incorporation (data not shown). Significant inhibition was obtained at a concentration as low as 10^{-8} M (fig. 3, day 2), greater inhibition being observed at 10^{-7} M and 10^{-6} M. 10^{-5} M Clomiphene, which was severely cytotoxic within 24 h, was not used for these and subsequent experiments.

The antiproliferative effect of antiestrogens was not limited to transformed lymphoid cells but was also observed in primary lymphocytes. Primary mouse splenocytes were induced into mitosis by exposure to concanavalin A (5 µg/ml) for 48 h at which time [^{3}H]thymidine incorporation (during a 6-h pulse) was 82,502 ± 2061 c.p.m./well (mean ± SD of triplicates) compared to 5669 ± 366 c.p.m./well (mean ± SD of triplicates) for unstimulated splenocytes. The mitogenic response to concanavalin A was significantly blunted by 10^{-6} M clomiphene as shown by a decrease in [^{3}H]thymidine incorporation (50,055 ± 2822 c.p.m./well; mean ± SD of triplicates) ($p < 0.001$). In separate experiments of identical design, concanavalin A-stimulated [^{3}H]thymidine incorporation (62,691 ± 3133 c.p.m./well; controls 10,893 ± 356 c.p.m./well) was

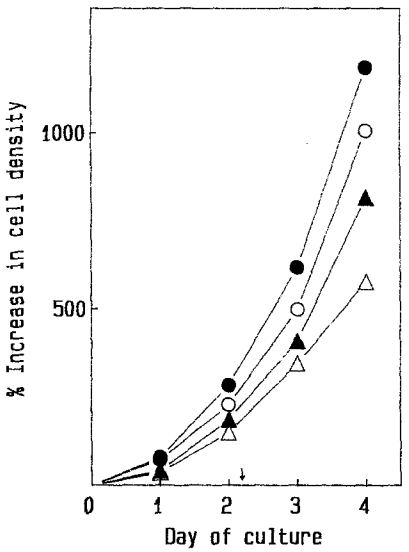

Figure 3. Dose response and reversibility of antiproliferative effect of clomiphene. Triplicate 25-cm^2 flasks of EL4 cells were set up at a starting density of about 300,000 cells/ml in charcoal-stripped RPMI 1640 medium. Forty-eight hours after clomiphene treatment (indicated by the arrow on day 2), cells in all flasks were transferred to RPMI 1640 medium without clomiphene. Cell proliferation was monitored by daily cell counts of triplicate flasks. Standard deviations were all < 5% of mean values. Control flasks (●) received 0.1% (v/v) dimethylsulfoxide alone. Test flasks received clomiphene at 10^{-8} M (○), 10^{-7} M (▲) and 10^{-6} M (△).

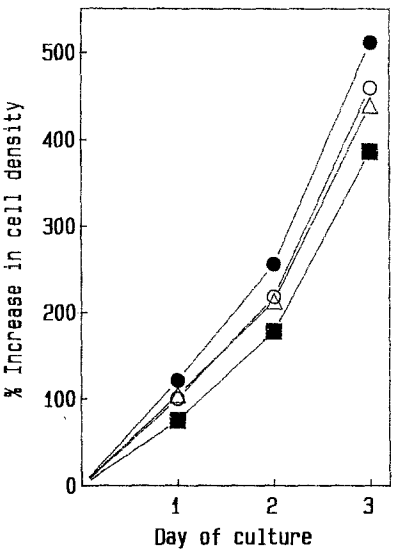

Figure 4. Interaction of linoleic acid and clomiphene on Raji cell proliferation. Raji cells in charcoal-stripped RPMI 1640 medium were inoculated into triplicate 25-cm^2 flasks at an initial density of about 300,000 cells/ml. Cell proliferation was monitored by daily cell counts and expressed as the mean percentage increase in cell density over the mean initial density for each group of triplicate flasks. Standard deviations were all < 5%. (○) Control flasks received 0.1% (v/v) dimethylsulfoxide and 10^{-5} M alpha-tocopherol. Test flasks received 10^{-6} M clomiphene (■); 10^{-5} M linoleic acid (●) and a combination of 10^{-6} M clomiphene and 10^{-5} M linoleic acid (△).

more profoundly inhibited by 10^{-6} M estradiol (20,958 ± 1811 c.p.m./well) than by 10^{-6} M clomiphene (38,410 ± 3120 c.p.m./well) ($p < 0.005$). An unexpected observation was the effectiveness of lower concentrations of estradiol: 10^{-7} M (24,271 ± 1238 c.p.m./well); 10^{-8} M (29,011 ± 1119 c.p.m./well); 10^{-9} M (34,116 ± 1238 c.p.m./well) (All values are mean ± SD of triplicates. $p < 0.001$ for differences between each concentration of estradiol and concanavalin A-stimulated value). Inhibition of [^{3}H]thymidine incorporation by pharmacological concentrations of estradiol was previously reported in phytohemagglutinin-stimulated rat splenocytes[31]. The absence of estrogen receptor in splenocytes implicates a non-receptor mechanism for this novel effect of estradiol. It is of interest that, unlike primary lymphocytes, EL4 cells were unaffected by 10^{-6} M estradiol (fig. 2).

Figure 3 also shows that the effect of antiestrogens on cell proliferation was reversible. When EL4 and Raji cells cultured in the presence of individual antiestrogens for 48 h were transferred to fresh medium without antiestrogens (indicated by the arrow in fig. 3), growth rates reverted towards normal. Reversibility was assessed by comparing growth rates (i.e. percentage increase in cell density over the previous day) of antiestrogen-treated cells, after removal of the agent, with those of control untreated cells after an identical transfer to fresh medium.

EL4 and Raji cells had no detectable estrogen receptor sites in the cytosol or crude nuclear pellet, and the antiproliferative effect of 10^{-6} M clomiphene on these cells was, unsurprisingly, not prevented by an equimolar concentration of estradiol (data not shown). The foregoing observations of a reversible, dose-dependent cytostatic action of antiestrogens on T- and B-lymphocytes lacking the estrogen receptor lend further support to the notion that certain effects of antiestrogens occur independently of the estrogen receptor and are probably not manifestations of estrogen antagonism at all. That the cytostatic effect did not result from nonspecific toxicity was shown by the inactivity of equimolar concentrations of steroids and a flavanoid, quercetin, known to inhibit the proliferation of tumor cells in culture[18].

Unsaturated fatty acids (oleic, linoleic and arachidonic) have been reported to inhibit [^{3}H]tamoxifen binding to the antiestrogen-binding protein in a crude preparation of rat liver microsomes[19]. We therefore tested their ability to modify the cytostatic effect of antiestrogens in this situation. Figure 4 shows that whereas Raji cells treated with 10^{-6} M clomiphene were growth-inhibited with respect to untreated cells ($p < 0.05$ on day 3), cells treated with a combination of 10^{-6} M clomiphene and 10^{-5} M linoleic acid had a growth rate not significantly different from the control. Thus, 10^{-5} M linoleic acid – a concentration known to inhibit antiestrogen binding in a cell-free system[19] – also completely abrogated the antiproliferative effect of 10^{-6} M clomiphene on Raji cells. Growth inhibition of EL4 cells by 10^{-6} M clomiphene was similarly prevented by 10^{-5} M linoleic acid or 10^{-5} M arachidonic acid (data not shown).

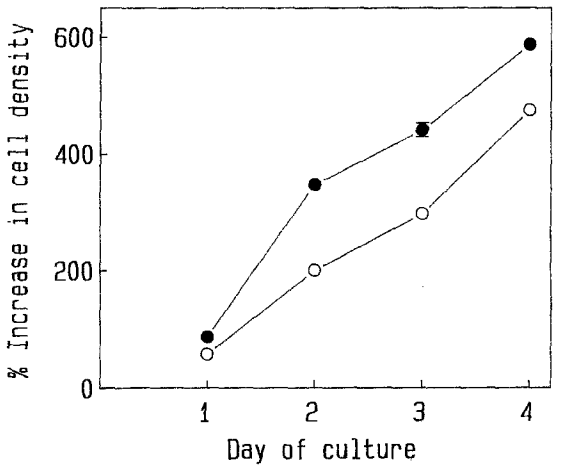

Figure 5. *A* Effect of charcoal-stripped medium on EL4 cell proliferation. EL4 cells in complete (●) or charcoal-stripped RPMI 1640 medium (○) were inoculated into two groups of triplicate 25-cm^2 flasks at an initial density of about 300,000 cells/ml. After 48 h of growth (on day 2), spent medium was removed and fresh complete medium replaced in all flasks. Cell proliferation, monitored by daily cell counts, is expressed as % increase over cell density just before transfer to fresh complete medium for days 3 and 4. *B* Growth rates of EL4 cells in charcoal-stripped RPMI 1640 medium in the absence (▲) and presence (△) of 10^{-8} M clomiphene.

The sensitivity of lymphoid cell proliferation to antiestrogens at concentrations as low as 10^{-8} M was an unexpected finding. All growth experiments in this study were performed in charcoal-stripped medium in which the growth rate of cells was lower than in complete medium (fig. 5A). Figure 5B shows that, in charcoal-stripped medium, the growth rate of EL4 cells in the presence of 10^{-8} M clomiphene progressively declined over 4 days compared to cells grown in the absence of clomiphene. This led us to examine the possibility that a mechanism for the sensitivity of lymphoid cells to low concentrations of antiestrogens may have been associated with some altered characteristic(s) of antiestrogen-binding sites induced by growth in charcoal-stripped medium. Scatchard analysis of the antiestrogen-binding protein from EL4 cells cultured for 6 days in charcoal-stripped medium or in complete medium showed that the affinity of [^{3}H]tamoxifen-binding sites in cells propagated in complete medium was lower (K_d = 1.68 nM ± 0.48 nM; mean ± SD of 6 analyses) than in cells propagated in charcoal-stripped medium (K_d = 0.54 nM ± 0.11 nM; mean ± SD of 6 analyses) ($p < 0.001$).

We next determined whether removal of endogenous fatty acids from the growth medium might have been at least partly responsible for the increased affinity of antiestrogen-binding sites induced by growth in charcoal-stripped medium. For this purpose, charcoal-stripped medium was supplemented with individual fatty acids at a final concentration of 10^{-5} M. EL4 cells were propagated for 6 days in such supplemented medium and compared with control cells grown in unsupplemented charcoal-stripped medium. Scatchard analyses showed that stearic acid and oleic acid did not significantly change the affinity of [^{3}H]tamoxifen binding. In contrast, linoleic acid decreased the affinity (K_d = 1.24 nM ± 0.10 nM) ($p < 0.01$) as did gamma-linoleic acid (K_d = 3.13 nM ± 1.02 nM; control K_d = 1.78 nM ± 0.39 nM) ($p < 0.05$), while palmitic acid increased the affinity (K_d = 1.34 nM ± 0.14 nM; control K_d = 1.78 nM ± 0.31 nM) ($p < 0.05$). (All values are the mean ± SD of 4 analyses). In contrast to changes in affinity, the abundance of antiestrogen-binding sites did not vary significantly with the culture conditions examined.

The observed changes in affinity were unlikely to have resulted from direct interference of fatty acids with [^{3}H]tamoxifen binding since harvested cell pellets were washed repeatedly with large volumes of fatty acid-free medium prior to cellular disruption. Rather, these findings provide the first demonstration that the affinity of the intracellular antiestrogen-binding protein is subject to modulation in whole cells by physiological concentrations of unsaturated fatty acids.

The same fatty acids were also tested for their intrinsic effect on EL4 cell proliferation. For this purpose, triplicate flasks of EL4 cells were set up in charcoal-stripped medium containing 0.1% (v/v) dimethylsulfoxide and 10^{-5} M alpha-tocopherol (controls) or in the presence of 10^{-5} M fatty acid supplementation. Growth was monitored by cell counting as described in Methods. The growth effect of each fatty acid was expressed as the difference in percentage increase in cell density after 48 h between control and fatty acid-supplemented cells. Thus, negative values indicate growth inhibition and positive values growth stimulation. By this criterion, oleic (+ 16%), linoleic (+ 38%) and gamma-linoleic (+ 138%) were growth stimulatory while palmitic (− 17%) and stearic (− 58%) were growth inhibitory.

The ultimate biological significance of these observations is at present unclear. Previous work has indicated that the antiestrogen-binding protein possesses a ligand-binding site for antiestrogens and an allosteric site for unsaturated fatty acids which decrease the affinity of antiestrogen binding[20]. It is possible that a natural class of ligands exists whose occupancy of the ligand-binding site leads to physiological restraint of cell proliferation. Oxysterols have now been reported to bind to the antiestrogen-binding site and may fulfill such a role[21,22].

It may be speculated that the antiestrogen-binding protein has some, as yet unknown, ligand-activated function(s). Changes in its binding affinity such as those described here, may be a physiological mechanism for regulating its function. We postulate that allosteric interaction of unsaturated fatty acids with the intracellular antiestrogen-binding protein leads to some conformational alteration of the ligand-binding site which we have detected here as an affinity change. Decreased affinity of binding, in turn, impedes binding of antiestrogens (and possibly their endogenous counterparts) to the ligand-binding site. Such a sequence would be consistent with our observations that cellular proliferation was slower in

charcoal-stripped medium, that unsaturated fatty acids were growth stimulatory and that linoleic acid reversed the antiproliferative effect of antiestrogens. Fatty acids may thus influence lymphoid cell proliferation, in part, by decreasing binding of an endogenous cytostatic ligand(s) to the antiestrogen-binding site.

Although the choice of lymphoid cells for these studies may appear unusual, antiestrogens have been reported to alter circulating lymphocyte concentrations, to modify a variety of lymphocyte functions such as natural killer activity and Fc receptors for IgG, and to suppress lectin-induced mitogenesis[23-31]. The changes induced, however, have been inconsistent and there is little agreement on what they signify. Nonetheless, two case reports of human lymphomas that responded to tamoxifen treatment[32,33] suggest that the present findings may have wider implications for this class of drugs. On a more fundamental level, they raise new and potentially interesting questions about the molecular mechanisms of antiestrogen action in nonestrogen target cells.

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Effects of a new cholecystokinin antagonist (GE 410) on the smooth muscle of the guinea pig ileum

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Summary. Suc-Tyr-(SE)-Met-Gly-Trp-Met-Asp-β-phenethylamide (GE 410) competitively antagonized the contractions of smooth muscle strips from guinea pig ileum ($pA_2 = 7.6$, $n = 0.95$) induced by cholecystokinin-octapeptide (CCK8). GE 410 inhibited the electrically-induced cholinergically mediated contractile responses and the [^{3}H]ACh release in the ileum, as well as the CCK-stimulated electrical contractile responses and the [^{3}H]ACh release in the cholinergic nerve terminals. The results suggest the existence of CCK-receptors not only in the smooth muscles but also on the neurons.

Key words. Cholecystokinin octapeptide; cholecystokinin antagonist (GE 410); [^{3}H]ACh release; ileac smooth muscle.