Interaction of unsaturated fatty acids with anti-oestrogen-binding sites

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Specific high-affinity binding sites for non-steroidal anti-oestrogens such as tamoxifen have been identified in many animal and human tissues. The function of these binding sites and the nature of their endogenous ligands are currently unknown. Our laboratory has previously reported that unsaturated fatty acids at micromolar concentrations inhibited [^3H]tamoxifen binding to the anti-oestrogen-binding sites in rat liver, raising the possibility that fatty acids might represent endogenous ligands for these sites. These studies have now been extended to examine the mechanism by which fatty acids inhibit [^3H]tamoxifen binding to the anti-oestrogen-binding site. Saturation analysis revealed that increasing concentrations of oleic acid progressively decreased the apparent binding affinity of these sites for [^3H]tamoxifen without decreasing the total number of binding sites; however, the apparent dissociation constant did not vary linearly with the prevailing oleic acid concentration, suggesting that the inhibition of [^3H]tamoxifen binding by fatty acid was not competitive in nature. Kinetic studies of [^3H]tamoxifen binding showed that oleic acid did not affect the rate of association, but increased the rate of dissociation of [^3H]tamoxifen from the anti-oestrogen-binding site; the latter finding would not be expected if oleic acid acted as a competitive inhibitor. Furthermore, incubation of a rat microsomal fraction with [^3H]oleic acid in the absence and presence of excess non-radioactively labelled tamoxifen also failed to demonstrate direct competition between oleic acid and tamoxifen for the same binding site. It is concluded that oleic acid, and presumably other unsaturated fatty acids, do not compete for the anti-oestrogen-binding site and probably reduce its tamoxifen-binding affinity by some other mechanism, such as perturbation of the lipid environment of the binding site. The biological significance of this interaction of unsaturated fatty acids with the anti-oestrogen-binding site remains to be elucidated.

INTRODUCTION

Specific high-affinity binding sites for the triphenyl-ethylene anti-oestrogens such as tamoxifen, distinct from oestrogen receptors, have been identified in many normal animal and human tissues (Sutherland *et al.*, 1980; Gulino & Pasqualini, 1982; Faye *et al.*, 1983; Kon, 1983; Sudo *et al.*, 1983) as well as in malignant cells (Miller & Katzenellenbogen, 1983; Watts *et al.*, 1984; Kon, 1985). The function of these binding sites is currently unknown, and their possible role in mediating the biological effects of the non-steroidal anti-oestrogens remains controversial (Miller & Katzenellenbogen, 1983; Murphy & Sutherland, 1983; Sheen *et al.*, 1985). It has been proposed that endogenous ligands for these sites may exist (Clark *et al.*, 1983; Murphy *et al.*, 1984), and several laboratories have attempted to identify ligands for these sites by looking for endogenous substances capable of inhibiting [^3H]tamoxifen binding to these sites. Murphy *et al.* (1985), for example, demonstrated that oxygenated metabolites of cholesterol inhibited [^3H]tamoxifen binding to anti-oestrogen-binding sites in chicken liver, whereas Brandes *et al.* (1985) reported that histamine antagonists competed for these sites in rat liver and suggested that histamine or histamine-like substances might represent endogenous ligands. Our laboratory recently showed that unsaturated fatty acids at micromolar concentrations were also capable of inhibiting

[^3H]tamoxifen binding to these sites (Hwang, 1986). It would be premature, however, to postulate that any of these substances might represent endogenous ligands, since none has been shown to bind directly to the anti-oestrogen-binding sites. Indeed, there are no studies to date on the mechanisms by which these various substances inhibit [^3H]tamoxifen binding. The studies reported here were carried out to examine the mechanism by which unsaturated fatty acids inhibit [^3H]tamoxifen binding to these sites.

MATERIALS AND METHODS

Chemicals

[*N*-methyl- ^3H]Tamoxifen (87 Ci/mmol) was obtained from Amersham International and stored in ethanol at -20°C protected from light. [^3H]Oleic acid (35 Ci/mmol) was obtained from New England Nuclear and stored under similar conditions. Tamoxifen citrate, diethylstilboestrol and fatty acids were from Sigma Chemical Co. Bio-Gel A-0.5 m (10% agarose) was from Bio-Rad. All other chemicals were of analytical grade and were purchased from conventional sources.

Tissue preparation

A 20000 *g* supernatant from rat liver, which contained anti-oestrogen-binding sites, was prepared and stored

Trivial names used: tamoxifen, (Z)-2-[p-(1,2-diphenylbuten-1-yl)phenoxy]-*NN*-dimethylethylamine; oleic acid, *cis*-9-octadecenoic acid.

as previously described (Hwang, 1986). Appropriate dilutions were made with ice-cold buffer [10 mM-Tris/HCl/1.5 mM-EDTA/20 mM-sodium molybdate/10% (v/v) glycerol, pH 7.5 at 4 °C] for the various studies described below.

Ligand-binding assay

Unless otherwise stated, this was carried out as described previously (Hwang, 1986).

Saturation analysis of [³H]tamoxifen binding in the absence and presence of fatty acid

Saturation analysis was carried out by incubating the rat liver 20000 g supernatant (0.5 ml; protein concentration 0.7 mg/ml) with various concentrations of [³H]tamoxifen (0.15–5.0 nM) for 16 h at 4 °C in the absence or presence of 10, 20, 30, and 40 μM-oleic acid. To terminate the assay, 0.25 ml of dextran-coated charcoal [10 mM-Tris/HCl/10% (v/v) glycerol/0.25% Norit A/charcoal/0.025% dextran T70, pH 7.5 at 4 °C] was added. The charcoal was pelleted by centrifugation at 1500 g for 15 min and the supernatants were counted for radioactivity as previously described (Hwang, 1986). Non-specific binding was determined by parallel incubations in the presence of a 500-fold molar excess of non-radioactively labelled tamoxifen. For comparison, a similar analysis was carried out using 1, 2, 4 and 8 nM non-radioactively labelled tamoxifen in place of oleic acid.

Association and dissociation studies

For these studies, which were carried out at 4 °C, the 20000 g rat liver supernatant was diluted to a final protein concentration of 0.5–1.0 mg/ml with buffer. Diethylstilboestrol was added to a final concentration of 1 μM to eliminate binding to oestrogen receptors.

For the association studies, 4.0 ml of rat liver supernatant was mixed with oleic acid (dissolved in 200 μl of ethanol) to give final concentrations of 0, 25 or 50 μM. The reaction was initiated by the addition of [³H]tamoxifen to a final concentration of approx. 1.5 nM. Aliquots (550 μl each) of the incubation mixture were removed at 20 s intervals up to 120 s and added to 250 μl of dextran-coated charcoal to terminate the reaction. Separation of bound from free ligand and determination of non-specific binding were carried out as described above. Specific binding was computed for each time point. To facilitate the determination of the association rate constant, the amount of [³H]tamoxifen added in each incubation approximately equalled the total binding sites present. Under these conditions, assuming that dissociation is negligible over brief time periods, the association rate constant (k_{+1}) can be obtained from the following second-order reaction equation:

$$1/(B_0 - B_T) - 1/B_0 = k_{+1}t$$

where t is time in seconds, B_0 is the total binding-site concentration and B_T is the concentration of binding sites occupied by [³H]tamoxifen at time t . By plotting $1/(B_0 - B_T)$ against t , k_{+1} is given by the slope. The number of total binding sites present (B_0) was determined by measuring specific binding after 16 h of incubation at 4 °C with saturating amounts of [³H]tamoxifen in the presence and absence of excess non-radioactively labelled tamoxifen.

For the dissociation studies, the diluted 20000 g liver supernatant was incubated for 16 h at 4 °C with 1 nM-[³H]tamoxifen in the absence or presence of various concentrations of oleic acid (5, 15 and 25 μM). At zero time a 2 500-fold excess of non-radioactively labelled tamoxifen was added. Aliquots (550 μl) of the incubation mixture were taken at 2, 5, 15, and 20 min and treated with 250 μl of dextran-coated charcoal. Total and specific binding was determined for each time point as described above. Since a large excess of unlabelled tamoxifen was added to prevent reassociation of [³H]tamoxifen, the dissociation reaction becomes first-order with respect to the concentration of the binding-site-[³H]tamoxifen complex. A plot of the specifically bound [³H]tamoxifen against time gives the time necessary for 50% of the ligand to dissociate from the binding sites. If this time is represented by $t_{1/2}$, the dissociation rate constant (k_{-1}) can be calculated from the relationship:

$$k_{-1} = \ln 2/t_{1/2}$$

Gel filtration

A 1 ml sample of rat liver 20 000 g supernatant diluted 10-fold with buffer (final protein concentration 0.7 mg/ml) was incubated at 4 °C for 16 h with approx. 250000 c.p.m. of [³H]tamoxifen (2.5 nM) in the absence or presence of 2.5 μM-tamoxifen or 500 μM-oleic acid. Chromatography was carried out on a Bio-Gel A-0.5 m column (10% agarose; 1.6 cm × 30 cm) equilibrated with 10 mM-Tris/HCl (pH 7.5)/1.5 mM-EDTA/20 mM-sodium molybdate/10% (v/v) glycerol. Fractions (2.6 ml) were collected and counted for radioactivity. An identical experiment was carried out with [³H]oleic acid (6 nM) in place of [³H]tamoxifen.

Other procedures

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Other studies were carried out as described in the appropriate Figure legends and Tables.

RESULTS AND DISCUSSION

Fig. 1(a) shows the Scatchard (1949) analysis of [³H]tamoxifen binding to the anti-oestrogen-binding site in the absence or presence of various concentrations of oleic acid, which has been chosen as a representative unsaturated fatty acid for these studies. In the absence of oleic acid, the plot indicated a single class of binding sites with an equilibrium dissociation constant of 0.77 nM. The presence of oleic acid did not significantly alter the number of binding sites (horizontal intercept), but clearly decreased the binding affinity (slope) in a dose-dependent manner. In the presence of 10, 20, 30 and 40 μM-oleic acid, the apparent equilibrium dissociation constant increased from 0.77 nM to 1.00, 1.14, 2.43 and 3.56 nM respectively. If the change in the apparent binding affinity for [³H]tamoxifen was due to competition by oleic acid, then the change in the apparent dissociation constant should fit the expression:

$$K'_d = K_d (1 + I/K_i)$$

where K_d is the dissociation constant for [³H]tamoxifen in the absence of oleic acid, K'_d the apparent dissociation constant for [³H]tamoxifen in the presence of oleic acid,

Fig. 1. Scatchard (1949) analysis of [^3H]tamoxifen binding to anti-oestrogen-binding sites of rat liver

(a) Rat liver 20000 *g* supernatant (0.5 ml; protein concentration 0.7 mg/ml) was incubated for 16 h at 4 °C with various concentrations of [^3H]tamoxifen (0.15–5.0 nM) in the absence (○) or presence of 10 μM (●), 20 μM (△), 30 μM (▲) or 40 μM (▽) oleic acid. Separation of bound from free ligand and determination of non-specific binding were carried out as described in the Materials and methods section. Results represent the means for five separate experiments. The straight lines were obtained by linear regression analysis. Inset: the apparent equilibrium dissociation constant K_d' (nM) was plotted against oleic acid concentration. (b) A similar study performed (in duplicate) in the absence (○) or presence of 1 nM (●), 2 nM (△), 4 nM (▲) or 8 nM (▽) non-radioactive tamoxifen.

I the concentration of oleic acid and K_i the dissociation constant for oleic acid. The relation predicts that, if oleic acid competes directly with [^3H]tamoxifen for the anti-oestrogen-binding site, K_d' should be a linear

Fig. 2. Measurement of the rate of association of [^3H]tamoxifen with the anti-oestrogen-binding sites of rat liver

The association studies were carried out as described in the Materials and methods section. (a) Shows the amount of specifically bound [^3H]tamoxifen as a function of time in the absence of (●) or presence of 25 μM (○) or 50 μM (△) oleic acid. (b) Plots the function $1/(B_0 - B_t)$ against time, where B_0 is the concentration (nM) of total binding sites and B_t is the concentration (nM) of binding sites occupied by [^3H]tamoxifen at time t . The association rate constants were obtained from the slopes of the plots, which were drawn by linear-regression analysis. The results show the means of duplicate determinations in a representative experiment.

function of oleic acid concentration (I). This is clearly not the case (Fig. 1a, inset), suggesting that some mechanism other than competitive inhibition is involved in the inhibition of [^3H]tamoxifen binding by fatty acid. On the other hand, when saturation analysis was carried out in the presence of 0, 1, 2, 4 and 8 nM non-radioactive tamoxifen, K_d' varied linearly with tamoxifen concentration (Fig. 1b, inset). This is expected, since tamoxifen competes directly with [^3H]tamoxifen for the same binding site.

To determine whether the increase in the apparent equilibrium dissociation constant seen in the presence of oleic acid is due to a decrease in the association rate or an increase in the dissociation rate, or both, kinetic studies were carried out. Fig. 2 shows the results of a representative experiment performed to determine the association rate constant. Fig. 2(a) shows that, for the first 2 min, specific binding of [^3H]tamoxifen increased linearly with time and that the association rate was not materially affected by the presence of 25 or 50 μM oleic acid, concentrations which are known to reduce binding

Fig. 3. Measurement of the rate of dissociation of bound $[^3\text{H}]$ tamoxifen from the anti-oestrogen-binding site of rat liver

The dissociation studies were carried out as described in the Materials and methods section. The amount of specifically bound $[^3\text{H}]$ tamoxifen in the absence (●) or presence of $5\ \mu\text{M}$ -(○), $15\ \mu\text{M}$ -(△), or $25\ \mu\text{M}$ -(▲) oleic acid is plotted against time. The results represent the mean of duplicate determinations. Half-times of dissociation were determined by linear-regression analysis.

significantly at equilibrium (Hwang, 1986). Fig. 2(b) shows the plots for the determination of the association rate constants in the absence and presence of oleic acid. The rate of association for brief reaction times, during which dissociation is negligible, may be considered as that of a second-order irreversible reaction, and determined as described in the Materials and methods section. The association rate constants in the presence of 0, 25 and $50\ \mu\text{M}$ -oleic acid, obtained by measuring the slopes of the plots in Fig. 2, were 8.0×10^5 , 8.0×10^5 and $7.5 \times 10^5\ \text{M}^{-1} \cdot \text{s}^{-1}$ respectively. From four separate experiments the association rate constants (mean $\pm$ S.D.) in the presence of 0, 25 and $50\ \mu\text{M}$ -oleic acid were 8.1 ± 0.6 , 9.3 ± 0.9 and $9.0 \pm 1.3 \times 10^5\ \text{M}^{-1} \cdot \text{s}^{-1}$ respectively; the differences were not statistically significant ($P > 0.05$).

Fig. 3 shows the dissociation of $[^3\text{H}]$ tamoxifen from the anti-oestrogen-binding site as a function of time. The dissociation appeared to be monophasic up to 20 min, both in the absence and in the presence of 5, 15 or $25\ \mu\text{M}$ -oleic acid. In the absence of oleic acid, the time required for 50% of specifically bound $[^3\text{H}]$ tamoxifen to dissociate ($t_{1/2}$) was 18.5 min, which corresponded to a dissociation rate constant of $6.2 \times 10^{-4}\ \text{s}^{-1}$. In the presence of 5, 15 and $25\ \mu\text{M}$ -oleic acid, dissociation was clearly accelerated with $t_{1/2}$ values of 15.0, 10.5 and 9.5 min respectively. The corresponding dissociation rate constants were $7.7 \times 10^{-4}\ \text{s}^{-1}$, $11.0 \times 10^{-4}\ \text{s}^{-1}$ and $12.2 \times 10^{-4}\ \text{s}^{-1}$ respectively.

Therefore it would appear that oleic acid increases the dissociation rate of $[^3\text{H}]$ tamoxifen from the anti-oestrogen-binding site without significantly affecting the association rate. The fact that the dissociation rate

Fig. 4. Gel filtration of rat liver 20000 *g* supernatant prelabelled with $[^3\text{H}]$ tamoxifen or $[^3\text{H}]$ oleic acid

(a) A 1 ml sample of rat liver 20000 *g* supernatant (protein concentration 0.7 mg/ml) was incubated at 4°C for 16 h with approx. 250000 c.p.m. (2.5 nM) of $[^3\text{H}]$ tamoxifen in the absence (○) or presence of $2.5\ \mu\text{M}$ -tamoxifen (●) or $500\ \mu\text{M}$ -oleic acid (△). Chromatography was carried out on a Bio-Gel A-0.5 m column as described in the Materials and methods section. Fractions (2.6 ml) were collected and counted for radioactivity. (b) As above, except that $[^3\text{H}]$ oleic acid (6 nM) was incubated with the rat liver supernatant in the absence (○) or presence (●) of $25\ \mu\text{M}$ -tamoxifen.

changed in the presence of oleic acid also suggests that the inhibition of $[^3\text{H}]$ tamoxifen binding by oleic acid is not achieved through competitive inhibition. Had oleic acid acted purely as a competitive inhibitor, the dissociation rate would have remained unchanged in the presence of the fatty acid.

To determine more directly whether fatty acids bind to the anti-oestrogen-binding site, $[^3\text{H}]$ oleic acid (2.5 nM) was incubated with the 20000 *g* liver supernatant in the presence or absence of a 1000-fold molar excess of non-radioactive tamoxifen ($2.5\ \mu\text{M}$). By using dextran-coated charcoal to separate bound from free radioactivity, no displaceable binding could be demonstrated. The radioactivity remaining in the supernatant (unadsorbed by charcoal) amounted to about 25% of the total added radioactivity in the absence as well as in the presence of tamoxifen. To exclude the possibility that the charcoal could have stripped off bound $[^3\text{H}]$ oleic acid from the anti-oestrogen-binding site, thereby obscuring any specific binding, the gel-filtration experiments depicted in Fig. 4 were carried out. Fig. 4(a) shows the

Table 1. Concentrations of *cis*- and *trans*-unsaturated fatty acids required for half-maximal inhibition of [³H]tamoxifen binding to rat liver anti-oestrogen-binding sites

[³H]Tamoxifen binding assays were set up as described previously (Hwang, 1986) in the absence and presence of various concentrations of fatty acids. The concentrations of fatty acids giving half-maximal inhibition of specific binding (IC₅₀) were read from the inhibition curves.

Fatty acid		IC ₅₀ (μM)
Trivial name	Systematic name	
Oleic	<i>cis</i> -9-Octadecenoic	50
Elaidic	<i>trans</i> -9-Octadecenoic	270
<i>cis</i> -Vaccenic	<i>cis</i> -11-Octadecenoic	48
<i>trans</i> -Vaccenic	<i>trans</i> -11-Octadecenoic	170
Erucic	<i>cis</i> -13-Docosenoic	60
Brassicic	<i>trans</i> -13-Docosenoic	> 500*

* Maximum concentration tested.

distribution of radioactivity when the 20000 g rat liver supernatant was preincubated with [³H]tamoxifen and then fractionated on Bio-Gel A-0.5 m. The peak at the void volume (V₀) was abolished by the presence of excess non-radioactive tamoxifen (present in 1000-fold molar excess, 2.5 μM), indicating that the anti-oestrogen-binding site emerged at the void-volume peak. As expected, the presence of 500 μM-oleic acid also abolished this peak; this concentration of oleic acid is known to inhibit completely [³H]tamoxifen binding to the anti-oestrogen-binding site. When the same experiment was carried out using [³H]oleic acid in place of [³H]tamoxifen, three peaks of radioactivity were observed, with the largest peak emerging at the void volume (Fig. 4b). The void-volume peak, however, did not appear to represent radioactivity bound to the anti-oestrogen-binding site because it remained unchanged in the presence of a 4000-fold molar excess of non-radioactive tamoxifen (25 μM), an amount known to be far in excess of that required to saturate all the anti-oestrogen-binding sites present. This observation clearly indicates that although [³H]oleic acid may bind to several different macromolecules in the liver supernatant, as suggested by the several peaks of radioactivity seen in gel filtration, the anti-oestrogen-binding site is not one of these. The failure of non-radioactive tamoxifen to displace [³H]oleic acid emerging at the void volume cannot be due to [³H]oleic acid having a higher affinity for the anti-oestrogen-binding site, since it has been previously demonstrated that oleic acid is only about 0.01% as potent at non-radioactive tamoxifen in displacing [³H]tamoxifen from this site (Hwang, 1986).

It is clear from the data presented above that oleic acid, and presumably other unsaturated fatty acids, do not compete with tamoxifen for the anti-oestrogen-binding site. Among alternative mechanisms which may explain the inhibition of [³H]tamoxifen binding by unsaturated fatty acids, one possibility is that fatty acids may alter the binding affinity of the anti-oestrogen-binding sites by perturbing the lipid environment of these binding sites. Several observations provide indirect support for this proposal. Firstly, it is currently believed

that the anti-oestrogen-binding sites are located in microsomal membranes (Sudo *et al.*, 1983), which, like other cell membranes, have a lipid bilayer as part of their structure. Secondly, it is known that fatty acids intercalate readily into the lipid bilayers of cell membranes. This, in turn, is believed to result in alterations in membrane structure and in the function of membrane-bound proteins such as receptors and enzymes (Karnovsky *et al.*, 1982; Stubbs & Smith, 1984; Spector & Yorek, 1985). In this regard, unsaturated fatty acids have been noted to be generally more effective than saturated fatty acids and *cis*-unsaturated fatty acids more effective than *trans*-unsaturated fatty acids in altering membrane structure and function. There appears to be a similar order of potency among fatty acids with regard to their inhibitory effect on [³H]tamoxifen binding. Unsaturated fatty acids, for example, have been shown to be much more potent than saturated fatty acids in inhibiting [³H]tamoxifen binding to the anti-oestrogen-binding site (Hwang, 1986). A comparison of several unsaturated fatty acids (Table 1) also revealed that *cis*-unsaturated fatty acids are more effective than *trans*-unsaturated fatty acids in inhibiting tamoxifen binding. The parallel between the effectiveness of various fatty acids in perturbing membrane lipid structure and the potency in inhibiting [³H]tamoxifen binding suggests that the two effects may possibly be linked. This possibility is given some support by the studies of Hill *et al.* (1983), who specifically examined the interaction between ¹⁴C-labelled fatty acids and rat liver microsomal membranes. They noted that the microsomal membranes did not contain high-affinity saturable binding sites for fatty acids and that the 'binding' of fatty acids by these membranes was a non-saturable process which could best be explained by partitioning into the lipid phase. This is also a likely explanation for our observation that when [³H]oleic acid was preincubated with the rat liver supernatant and gel filtration carried out as described in Fig. 4, the void-volume peak of radioactivity was not abolished by the presence of a large molar excess of non-radioactive oleic acid (result not shown).

The possibility that oleic acid was metabolized during incubation and that its inhibitory effect on [³H]tamoxifen binding was exerted through a metabolite was explored. [³H]Oleic acid was incubated with the 20000 g rat liver supernatant under the same conditions as the studies described previously. The incubation mixture was extracted with diethyl ether, the extract was dried under N₂, redissolved in chloroform and fractionated by t.l.c. with three different solvent systems: hexane/diethyl ether/acetic acid (70:30:1, by vol.), benzene/acetone (4:1, v/v), and diethyl ether/benzene/ethanol/acetic acid (200:250:10:1, by vol.). In all three systems it was observed that about 90–93% of the total radioactivity migrated with a mobility identical with that of oleic acid. A small peak of radioactivity, comprising 1–4% of the total radioactivity, was observed migrating ahead of oleic acid. This peak was not seen when [³H]oleic acid was incubated with buffer alone and could indeed represent a metabolite of oleic acid. However, additional studies using material extracted from parallel incubations of unlabelled oleic acid with liver supernatant indicated that this fast-migrating peak had no inhibitory activity on [³H]tamoxifen binding. These findings suggest that oleic acid itself, and not one of its metabolites, is responsible for the inhibition of [³H]tamoxifen binding.

Although these observations were consistent with the suggestion that unsaturated fatty acids inhibit [^3H]tamoxifen binding by a non-competitive mechanism, possibly via perturbation of membrane structure, the evidence must be viewed as tentative, because the binding studies were carried out with crude liver extracts and not with purified anti-oestrogen-binding sites. A direct demonstration of the mechanism of fatty acid inhibition of [^3H]tamoxifen binding will require the isolation of the anti-oestrogen-binding sites.

The biological significance of the effect of fatty acids on [^3H]tamoxifen binding is at present unknown, and speculation is difficult because the function of the anti-oestrogen-binding site remains obscure. It is worth noting, however, that Brandes & Bogdanovic (1986) reported studies suggesting that this site may mediate, at least in part, the antiproliferative effect of a synthetic diphenylmethane derivative, *NN*-diethyl-2-[(4-phenylmethyl)phenoxy]ethanamine hydrochloride. This compound binds with high affinity to the anti-oestrogen-binding site but does not bind to the oestrogen receptor. Nevertheless, it inhibits oestrogen-stimulated growth of the rat uterus *in vivo* (Brandes & Bogdanovic, 1986) and the growth of MCF-7 human breast-cancer cells *in vitro* (Brandes, 1984). These findings suggested to Brandes & Bogdanovic (1986) that the anti-oestrogen-binding site might possibly be involved in the regulation of cell growth and proliferation. Similarly, the studies by Runge *et al.* (1986) also suggested that the inhibitory effect of tamoxifen and its metabolites on the growth of human ovarian cancer cells in culture may be mediated by the anti-oestrogen-binding site and not by the oestrogen receptor. It has long been known from both experimental and epidemiological studies that unsaturated fatty acids enhance the growth of a number of animal and human tumours (Committee on Diet, Nutrition and Cancer, National Research Council, 1982). The possibility clearly exists that the effects of fatty acids on cellular growth may be related to their interaction with the anti-oestrogen-binding site.

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