Study of the in-vivo antioestrogenic action of N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine HCl (DPPE), a novel intracellular histamine antagonist and antioestrogen binding site ligand

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Summary. N,N-Diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine HCl (DPPE) binds with high affinity to the antioestrogen binding site (AEBS), but not to the oestrogen receptor. There is an association of AEBS with a novel intracellular histamine receptor (H_{1C}) of micromolar affinity through which histamine acts as a second messenger. An optimal dose of 4 mg DPPE/kg antagonized the uterine growth-stimulating effects of oestradiol in immature oophorectomized rats. Unlike tamoxifen, DPPE alone was not a partial agonist, but decreased uterine size and weight below control values at concentrations between 0.1 and 75 mg/kg. DPPE also antagonized oestradiol-stimulated uterine growth at 72 h; the inhibition observed was not significantly different from that seen with tamoxifen. Oestradiol-treated animals receiving the combination of DPPE (4 mg/kg) + low dose tamoxifen (0.04 mg/kg) for 72 h had significantly smaller uteri than did those receiving the same dose of DPPE or tamoxifen alone. Histologically, either DPPE or tamoxifen antagonized oestradiol stimulation of eosinophil migration and glandular epithelial proliferation; the latter inhibition was significantly greater for DPPE + tamoxifen (0.04 mg/kg) than for the same dose of DPPE or tamoxifen alone. Unlike tamoxifen, DPPE did not antagonize oestradiol stimulation of luminal epithelial proliferation, but in the presence of oestradiol, DPPE significantly decreased tamoxifen (0.65 mg/kg)-induced hypertrophy of the luminal epithelium. Based on these findings, we suggest that binding to the AEBS/intracellular histamine receptor is important to the action of antioestrogens.

Keywords: oestrogen; antioestrogen; antioestrogen binding site; histamine

Introduction

The effects of oestrogens on reproductive tract physiology appear to result from complex receptor interactions (Knowler & Beaumont, 1985), involving both classical and alternative (non-genomic) pathways (Tchernitchin, 1983), and to depend upon the activation of various intracellular second messengers such as cyclic AMP (Szego & Davis, 1967) and calcium (Pietras & Szego, 1975). Similarly, the antioestrogenic/antiproliferative effects of tamoxifen may be multifactorial in origin (Jordan *et al.*, 1981), since in addition to binding to the oestrogen receptor, this triphenylethylene derivative also interacts with microsomal antioestrogen binding sites (Murphy *et al.*, 1981) and antagonizes the action of calmodulin (Lam, 1984), protein kinase C (O'Brien *et al.*, 1986) and calcium (Greenberg *et al.*, 1987).

To determine a precise role for various pathways and their mediators in oestrogen/antioestrogen action, selective ligands are required. In this regard, a para-diphenylmethane derivative synthesized

in our laboratory, N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine HCl (DPPE), binds to antioestrogen binding sites with high affinity ($K_i = 65 \times 10^{-9}$ M) (Brandes & Hermonat, 1984). Unlike tamoxifen, DPPE does not bind significantly to oestrogen receptors in rat uterine cytosol (Brandes & Hermonat, 1984) or antagonize the action of calmodulin (Brandes *et al.*, 1986) or protein kinase C (Brandes *et al.*, 1988). Although DPPE competes for sites labelled by [3 H]-verapamil (Brandes *et al.*, 1987) its various actions cannot be attributed to calcium channel antagonism (Brandes *et al.*, 1987; Glavin & Brandes, 1988).

In vitro, DPPE is antiproliferative at lower (1–10 μ M) concentrations and cytotoxic at higher (> 10 μ M) concentrations against both oestrogen receptor-positive and oestrogen receptor-negative breast cancer cells (Brandes, 1984; Brandes *et al.*, 1985) and augments similar effects of tamoxifen (Brandes, 1984). In the immature, oophorectomized rat, DPPE alone has been demonstrated to decrease uterine weight below control values, unlike tamoxifen alone, which is a partial agonist. Like tamoxifen, however, DPPE antagonizes the growth-promoting effects of exogenous oestradiol (Brandes & Bogdanovic, 1986), despite the fact that it does not compete for oestrogen receptors (Brandes & Hermonat, 1984). These findings support an important role for antioestrogen binding sites in the mechanism of action of tamoxifen.

Studies of rat brain membranes (Brandes *et al.*, 1987) and human platelets (Brandes *et al.*, 1988; Saxena *et al.*, 1989; McNicol *et al.*, 1989) provide evidence that the DPPE/anti-oestrogen binding site is associated with an intracellular histamine receptor (H_{IC}) of micromolar affinity, which is different from the classical H_1 and H_2 receptors and the H_3 receptor (Schwartz *et al.*, 1986). By binding at this site, newly-formed histamine functions as a second messenger for multiple agonists mediating platelet aggregation (Saxena *et al.*, 1989) and is implicated as a mediator of growth (Brandes *et al.*, 1987). We have proposed that, by binding to antioestrogen binding sites, DPPE and other diphenylmethane-derivative ligands antagonize the action of histamine at the associated H_{IC} site (Brandes *et al.*, 1987), their antiproliferative action correlating with K_i values for [3 H]histamine binding (Brandes *et al.*, 1987, 1988). The in-vitro antagonism of cell growth by DPPE is significantly reversed by L-histidine and L-methionine (Brandes *et al.*, 1987), two amino acids involved in histamine metabolism, while in agonist-stimulated platelets the anti-aggregatory effect of DPPE is reversed in permeabilized, but not intact, cells by 0.1–10 μ M-histamine, suggesting an action of DPPE on an intracellular histamine target of micromolar affinity (Saxena *et al.*, 1989).

To elucidate further a role for an antioestrogen binding site/ H_{IC} receptor in antioestrogen action, we have studied oophorectomized immature female rats to assess dose-responses for DPPE and to compare the effects on uterine size and histology of DPPE alone with those of saline (control), oestradiol and tamoxifen alone. The effects of saline, DPPE, tamoxifen and combined DPPE + tamoxifen pretreatment on the uteri of similar rats receiving oestradiol for 72 h also have been assessed.

Materials and Methods

Animals

Immature (average 60–80 g) oophorectomized female Sprague-Dawley rats were obtained from Charles River, St Constant, Quebec, Canada. Animals were housed in cages of 4–6, fed water and chow *ad libitum* and exposed to a 12 h light:12 h dark schedule.

Drug preparation and route of administration

Oestradiol and tamoxifen (Sigma, St Louis, MO, USA) were dissolved in 50% ethanol before dilution in saline (0.154 M-NaCl). The final effective ethanol concentration for oestradiol was 18.5%, while that for tamoxifen was 15%. DPPE was synthesized as described previously (Brandes & Hermonat, 1984) and dissolved directly in saline.

All drugs were given by the i.p. route. Previous studies have shown that the serum concentrations resulting from i.p. or oral administration of tamoxifen are similar (Fromson *et al.*, 1973).

Experimental procedure

Groups of rats received either saline or DPPE in doses ranging from 1 to 32 mg/kg, given 1 h before oestradiol (100 µg/kg), daily for 3 days. At 72 h after the first injection, the animals were killed by decapitation, and the uteri were quickly removed by blunt dissection, stripped of excess tissue and weighed.

To assess the effects of DPPE administration on organs insensitive to the growth-promoting effects of oestradiol, the liver, spleen and kidneys were also removed; the wet weight of each organ was determined and compared with control (saline) values.

To assess the effects of saline, oestradiol (100 µg/kg), DPPE (4 mg/kg or 1.25×10^{-5} mol/kg) or tamoxifen (0.65 mg/kg, or 1.15×10^{-6} mol/kg) alone on uterine size and histology, groups of animals received the various agents once daily for 3 days. After dissection and weighing, uteri were stored in formalin until histological study.

The effects on uterine size and histology of saline, DPPE, tamoxifen, or tamoxifen + DPPE were compared when each was administered 1 h before oestradiol for 3 days. A higher dose of oestradiol (300 µg/kg) was chosen for these experiments in an attempt to obtain maximal stimulation for histological assessment, especially pertinent to eosinophil migration (Tchernitchin, 1983). To assess possible synergism between DPPE and tamoxifen, two doses of tamoxifen were used, an optimal dose of 0.65 mg/kg and a low dose of 0.04 mg/kg (7.1×10^{-8} mol/kg). The latter dose results in substantially decreased, although still significant, oestrogenic and antioestrogenic action in the rat uterus, compared with the optimal dose of tamoxifen (Allen *et al.*, 1980). The dose of DPPE remained constant (4 mg/kg) allowing comparison of additive effects of DPPE to the optimal and low doses of tamoxifen.

Histological studies

For histological studies, 4 paraffin wax-embedded transverse sections, cut at different levels, were prepared identically for all uteri, stained with haematoxylin and eosin, and assessed microscopically by one individual (G.R.H.).

For luminal epithelium, height (µm) and the number of mitotic figures were assessed. To measure accurately the height of luminal epithelium, only cells with the best orientation, showing no artefactual distortion on a thin basement membrane complex, were included. Such cells were found to be present in small strips and to be uniform in size and appearance. For glandular epithelium, the number of mitotic figures was assessed. In the luminal and glandular epithelia, the mitotic figure count per section included only those cells in which mitosis was definitely recognizable.

For each uterus, the total number of eosinophils (stroma plus circular muscle layer)/section was counted. Measurement of eosinophilia was confined to the entire endometrial stroma and circular muscle, as infiltration was noted to be most dense at the interface of these structures. Artefactual damage at the level of the serosa and longitudinal muscle occurred frequently enough to preclude accurate assessment; therefore estimation in these areas was not included.

In some experiments, microscopic cross-sectional area of uteri was roughly estimated by multiplying horizontal and vertical diameters, as measured by micrometer.

Statistical analysis

Effects on uterine weight. Significant differences in final body weight among treatment groups may of themselves influence uterine weight (Lyman & Jordan, 1985). However, the maximal difference observed in final body weight among the treatment groups was only 5%. Analysis of covariance, to adjust for final body weight, had no effect on statistical significance for any group. Therefore, analysis of variance of the unadjusted mean uterine wet weights was applied over all four treatment groups (Table 2), or all 6 treatment groups (Table 3), as well as over a sub-set of 3 treatment groups (Table 2) or of 5 treatment groups (Table 3) excluding the oestradiol (or saline + oestradiol) group, since the effect of oestrogen treatment on the uterine response was deemed predictable from previous results (Katzenellenbogen *et al.*, 1981; Brandes & Bogdanovic, 1986). Since all F ratios from the ANOVA were found to be significant at $P \leq 0.01$, Fisher's Restricted (Protected) Least Significant Difference test (Snedecor & Cochran, 1980) was then applied.

Effects on uterine histology. To determine significant effects among treatment groups on each parameter of uterine histology, analysis of variance (ANOVA) for multiple comparison protection, as above, and Fisher's Restricted L.S.D. test were used. For analysis of mitosis in luminal and glandular epithelium, as well as numbers of eosinophils, for which the standard deviations were proportional to the mean counts, indicating unequal variances, and the counts themselves were, in some groups, very low, a log transformation, $\ln(x + 0.05)$, was applied to stabilize the variances before the subsequent analysis.

Results

Dose-response of DPPE

The optimal daily dose of DPPE to antagonize oestradiol stimulation of uterine growth over 72 h was 4 mg/kg (Fig. 1), with lesser, but significant, effects at 2 mg/kg and 8 mg/kg. At 4 mg

DPPE/kg, the uterine wet weight was 77.2 ± 3.8 mg (mean $\pm$ s.e.m.), while that for controls was 99.4 ± 4.3 mg ($P < 0.001$). At doses below 2 mg/kg and above 8 mg/kg, DPPE did not significantly antagonize oestradiol. However, despite the bell-shaped dose-response curve for DPPE in the presence of oestradiol, when tested alone over a wide daily dosage range, DPPE did not increase uterine size above control (saline alone); on the contrary, at all dosages from 0.1 to 75 mg/kg, DPPE treatment resulted in significantly ($P < 0.05$) smaller uteri than in saline-treated controls (Fig. 2).

Fig. 1. Dose-response for DPPE to antagonize the growth-promoting effects of oestradiol (100 µg/kg) on the uteri of immature oophorectomized rats at 72 h. Values are mean $\pm$ s.e.m. for the no. of rats indicated.

Fig. 2. Effects of various doses of DPPE alone on uterine size of immature oophorectomized rats at 72 h. Values are mean $\pm$ s.e.m. for the no. of rats indicated.

DPPE treatment did not affect the wet weight of the liver, spleen or kidneys, compared with those of controls: liver, 4022 ± 220 mg vs 4050 ± 205 mg; spleen, 325 ± 38 mg vs 296 ± 19 mg; kidneys 991 ± 47 mg combined vs 1009 ± 25 mg combined.

Comparison of effects on uteri of DPPE, saline, oestradiol and tamoxifen alone

As shown in Table 1, the uteri of animals receiving DPPE were smallest in estimated cross-sectional area ($P < 0.02$ vs saline; $P < 0.001$ vs oestradiol or tamoxifen), and luminal epithelial height of uteri was also the lowest ($P < 0.001$ vs oestradiol or tamoxifen; NS vs saline). Luminal epithelial and glandular mitosis was significantly increased only in oestradiol-treated animals. Eosinophil content of uteri of DPPE- and saline-treated animals was zero, while that of oestradiol-treated animals was highest. Tamoxifen-treated animals also demonstrated some degree of uterine eosinophilia but the value was not significantly different from that of the oestradiol group because of the high standard errors.

Table 1. Effects after 72 h of saline, oestradiol, DPPE or tamoxifen alone on uterine size and histology of immature oophorectomized Sprague-Dawley rats

Group	No. of rats	Cross-sectional area (μm^2)*	Epithelial height (μm)	Mitotic figures/section		No. of eosinophils/section (stroma + circular muscle)
				Luminal epithelium	Glandular epithelium	
Saline	6	0.64 ± 0.04	12.7 ± 0.8	0.3 ± 0.1	0	0
Oestradiol	6	$2.43 \pm 0.35^{**}$	$29 \pm 1.3^{\dagger}$	3.0 ± 0.7	1.0 ± 0.3	21 ± 11
DPPE	6	$0.47 \pm 0.03^{***}$	11.2 ± 0.4	0	0	0
Tamoxifen	6	$0.95 \pm 0.05^{\ddagger}$	$32 \pm 1.8^{\ddagger}$	0	0	6 ± 3

Values are mean $\pm$ s.e.m.

*Estimate based on vertical and horizontal measurement by micrometer.

** $P < 0.001$ vs all other groups.

*** $P < 0.02$ vs saline; $P < 0.001$ vs tamoxifen.

$^{\dagger}P < 0.001$ vs saline.

$^{\ddagger}P < 0.001$ vs saline or DPPE.

Comparison of antagonism of oestradiol action on uteri at 72 h

The uteri of oestradiol-treated animals receiving the antioestrogens were all significantly smaller than those of the corresponding saline controls (Table 2). No significant difference in uterine wet weight was observed between DPPE and tamoxifen groups in the same set of experiments. A significant difference ($P < 0.05$) was observed for the combined DPPE + tamoxifen (0.65 mg/kg) group compared with DPPE alone, but not compared with tamoxifen (0.65 mg/kg) alone (Table 2A). When the tamoxifen dose was reduced (0.04 mg/kg) the value for combined DPPE + tamoxifen treatment did differ significantly ($P < 0.05$) from that of tamoxifen alone (Table 2B).

The effect of the various treatments on uterine histology is shown in Table 3. In the presence of oestradiol, DPPE-treated animals consistently had the lowest luminal epithelial height, but the value was significantly ($P < 0.001$) different only from that of animals receiving 0.65 mg tamoxifen/kg. Treatment with DPPE + 0.65 mg tamoxifen/kg resulted in a significantly higher epithelial height than in saline-treated animals ($P < 0.001$) but a significantly lower height than in animals treated with 0.65 mg tamoxifen/kg alone ($P < 0.05$).

Only the DPPE group failed to demonstrate significant inhibition of luminal epithelial mitosis. While a dose-response was observed for the two concentrations of tamoxifen, DPPE did not augment either dose to inhibit luminal epithelial mitosis. In contrast to its lack of inhibitory effect on

Table 2. Antagonism of oestradiol-stimulated (300 µg/kg) uterine growth at 72 h by saline, DPPE (4 mg/kg), tamoxifen (A, 0.65 mg/kg; B, 0.04 mg/kg) and DPPE + tamoxifen in immature oophorectomized Sprague-Dawley rats

Group	No. of rats	Uterine wet wt (mg)	% Decrease vs control	Significance*			
				Group 1	Group 2	Group 3	Group 4
A (1) Saline + oestradiol	7	146.3 ± 10.4	—	—	$P < 0.05$	$P < 0.01$	$P < 0.01$
(2) DPPE + oestradiol	8	124.5 ± 7.8	15	$P < 0.05$	—	n.s.	$P < 0.05$
(3) Tamoxifen + oestradiol	7	107.2 ± 6.6	27	$P < 0.01$	n.s.	—	n.s.
(4) DPPE + tamoxifen + oestradiol	8	98.2 ± 5.5	33	$P < 0.01$	$P < 0.05$	n.s.	—
B (1) Saline + oestradiol	22	128.0 ± 3.7	—	—	$P < 0.001$	$P < 0.001$	$P < 0.001$
(2) DPPE + oestradiol	24	105.8 ± 4.0	17.5	$P < 0.001$	—	n.s.	$P < 0.01$
(3) Tamoxifen + oestradiol	23	99.0 ± 3.4	23	$P < 0.001$	n.s.	—	$P < 0.05$
(4) DPPE + tamoxifen + oestradiol	27	90.5 ± 2.5	30	$P < 0.001$	$P < 0.01$	$P < 0.05$	—

Values are mean ± s.e.m.

*Fischer's Restricted Least Significant Difference test.

†Dosage of DPPE + tamoxifen is the same as either alone.

Table 3. Antagonism of the effects of oestradiol (300 µg/kg) at 72 h on uterine histology of immature, oophorectomized Sprague-Dawley rats by saline, DPPE (4 mg/kg), tamoxifen (0.65 or 0.04 mg/kg), and DPPE + tamoxifen

Group	No. of rats	Luminal epithelium		Glandular epithelium	No. of eosinophils/section
		Height (µm)	Mitotic figures/section	Mitotic figures/section	
(1) Saline	29	31.3 ± 0.31 ^a	1.28 ± 0.23 ^a	0.63 ± 0.14 ^a	40.2 ± 6.6 ^a
(2) DPPE	32	30.2 ± 0.40 ^a	0.93 ± 0.16 ^a	0.30 ± 0.07 ^b	21.5 ± 2.3 ^{bd}
(3) Tamoxifen (0.65 mg/kg)	7	38.8 ± 0.39 ^b	0.12 ± 0.05 ^b	0.04 ± 0.03 ^{bcd}	24.2 ± 2.2 ^{bd}
(4) Tamoxifen (0.04 mg/kg)	23	31.2 ± 0.40 ^a	0.42 ± 0.13 ^{ad}	0.32 ± 0.07 ^b	20.0 ± 1.9 ^{bd}
(5) DPPE + tamoxifen (0.65 mg/kg)	8	36.1 ± 0.55 ^{ac}	0.03 ± 0.02 ^c	0.03 ± 0.02 ^{bcd}	13.7 ± 1.0 ^{bce}
(6) DPPE + tamoxifen (0.04 mg/kg)	27	31.9 ± 0.50 ^a	0.35 ± 0.08 ^{ad}	0.14 ± 0.03 ^{bcd}	14.8 ± 1.3 ^{bce}

Values are mean ± s.e.m.

Values without a superscript letter in common are significantly different ($P < 0.05$ at least; see text for actual P values).

the oestradiol-stimulated luminal epithelium, DPPE, like tamoxifen, significantly antagonized mitotic activity in the glandular epithelium (Table 3). The combination of DPPE + 0.04 mg tamoxifen/kg was significantly more inhibitory to mitosis in glandular epithelium than was the

same dose of either alone ($P < 0.02$). However, the greatest inhibition of glandular epithelium was observed with 0.65 mg tamoxifen/kg.

A significant decrease in oestradiol-stimulated uterine eosinophilia was seen for DPPE and both tamoxifen alone groups compared with controls (Table 3), and the decrease was more marked with both the combined treatments.

Discussion

The results confirm our earlier observations that the selective antioestrogen binding site ligand DPPE decreases uterine size and weight when given alone, and antagonizes the effects of exogenous oestradiol on the uterus in the immature oophorectomized rat (Brandes & Bogdanovic, 1986). Previously, a single-ringed compound (BPEA) with affinity for antioestrogen binding sites, but not for oestrogen receptors, was reported to be inactive *in vivo* (Sheen *et al.*, 1985). However, the binding studies published for this compound reveal a potency for [^3H]tamoxifen binding in rat liver microsomes one order of magnitude lower (~ 500 nM) than that for DPPE (Brandes & Hermonat, 1984).

It may be argued that DPPE is not really antioestrogenic in the true sense, but simply exerts a generalized negative antiproliferative effect on cells and tissues, including the uterus, in the absence or presence of oestrogen. However, several findings make this unlikely: (a) unlike its inhibitory effect on both the oestradiol-stimulated and unstimulated uterus, DPPE administration under the same conditions had no significant effect on the wet weight of the liver, spleen and kidneys, all of which contain antioestrogen binding sites (Kon, 1983) but are not stimulated to proliferate by oestrogen; (b) despite decreasing uterine weight and size at concentrations between 0.1 and 75 mg/kg, DPPE significantly antagonized oestradiol effects on uterine growth only when administered at doses of 2, 4 and 8 mg/kg and not at higher doses of 16 and 32 mg/kg; (c) when given 1 h after oestradiol, DPPE previously was shown not to prevent uterine growth (Brandes & Bogdanovic, 1986); and (d) DPPE selectively antagonized the growth-promoting effects of oestradiol on the uterine glandular but not luminal epithelium.

Although the bell-shaped dose-response curve for DPPE in the presence of oestradiol suggests a partial agonist action, none was seen. The shape of the curve is similar to that observed for histamine reversal of the anti-aggregatory effects of DPPE in permeabilized platelets (Saxena *et al.*, 1989).

The significant decrease in uterine size and weight in immature oophorectomized rats treated with DPPE alone might suggest antagonism of small amounts of residual hormone, possibly of adrenal origin. In support of this suggestion, DPPE has been shown in rats to block the stress-induced rise in corticosterone (Glavin & Brandes, 1988), indicating a possible inhibitory effect on adrenal cortical function. Alternatively, like tamoxifen (Jordan *et al.*, 1981), DPPE could antagonize the pituitary-hypothalamic axis. Further studies are required to elucidate the exact mechanism by which DPPE causes uterine atrophy in oophorectomized animals.

While some effects of DPPE on uterine histology differ from, others are similar to, those of tamoxifen. For example, whereas tamoxifen inhibited oestradiol-stimulated luminal epithelial proliferation and, at its optimal dose (0.65 mg/kg), caused significant hypertrophy of luminal epithelium in the presence of oestradiol, DPPE had no such effect, although in the presence of oestradiol it significantly decreased tamoxifen-induced epithelial hypertrophy. On the other hand, it was observed that both DPPE and tamoxifen inhibited oestradiol-stimulation of glandular epithelial proliferation, with the combination of DPPE and low-dose (0.04 mg/kg) tamoxifen demonstrating significantly additive effects. The greater potency of the optimal dose of tamoxifen to inhibit glandular epithelium may result from its 12-fold higher affinity for antioestrogen binding sites compared with DPPE (Brandes & Hermonat, 1984). Finally, although both DPPE and tamoxifen alone antagonized oestradiol-stimulated uterine eosinophil content, their combined

inhibitory action on uterine eosinophilia was greatest. Whether this effect is due to an interaction at the level of the eosinophil itself (Tchernitchin, 1983), or an effect of DPPE and tamoxifen to alter the production of chemotactic or other factors in the uterus, remains to be determined.

In summary, while the actions of DPPE and tamoxifen differ both qualitatively and quantitatively in certain fundamental respects, nevertheless DPPE specifically antagonizes oestradiol stimulation of uterine growth, of eosinophil migration and of glandular epithelial proliferation. Moreover, the combination of DPPE and a low dose of tamoxifen is significantly more inhibitory than either alone. As DPPE interacts with antioestrogen binding sites/ H_{1C} receptors, but not with oestrogen receptors, this could indicate that binding to the former site(s) is also important to the overall mechanism of action of tamoxifen.

Some years ago, it was recognized that the ring (triphenylethylene) structure of tamoxifen is related to known oestrogens and the aliphatic constituent to known (H_1) antihistamines (Harper, 1967), but its effects to prevent implantation could not be mimicked by traditional H_1 antagonists (Harper, 1965). We suggest that, like DPPE, tamoxifen acts at the antioestrogen binding/ H_{1C} site, rather than at an H_1 extracellular site (Spaziani & Szego, 1959; Szego, 1965) to antagonize histamine. As previously demonstrated in the kidney (Maeyama *et al.*, 1985), oestrogen could activate histidine decarboxylase in the uterus, with newly formed histamine functioning as an intracellular second messenger to mediate some oestrogen action.

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