J. steroid Biochem. Vol. 27, No. 4–6, pp. 883–889, 1987
Printed in Great Britain.

0022-4731/87 $3.00 + 0.00

Proceedings of the VII International Congress on Hormonal Steroids (Madrid, Spain, 1986)

THE COMPLEXITY OF ANTI-ESTROGEN RESPONSES

J. R. PASQUALINI*, C. SUMIDA, N. A. GIAMBIAGI and B.-L. NGUYEN

C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26 Boulevard Brune, 75014 Paris, France

Summary—The actions and biological responses of anti-estrogens are a function of: the experimental conditions, the parameters, the organ and the animal species considered. Target tissues for estrogens in the guinea-pig during the perinatal period are interesting models to explore the action of anti-estrogens. The summary of the data indicates: (1) In the fetal uterus of guinea-pig in *in vivo* experiments (after injection to the maternal compartment) tamoxifen acts as a real agonist concerning growth, as a partial agonist concerning the stimulation of the progesterone receptor. (2) In *in vitro* experiments (in organ culture of fetal uterus or in isolated cells) anti-estrogens (tamoxifen or 4-hydroxy-tamoxifen) act as antagonists and also inhibit the effects provoked by estrogens. (3) In the uterus and vagina of newborn guinea-pigs, tamoxifen and its derivatives: 4-hydroxytamoxifen and *N*-desmethyltamoxifen act as real agonists concerning the uterotrophic and vaginotrophic effects, and also stimulate the amount of DNA per organ, but concerning the progesterone receptor in the uterus, in the short treatment anti-estrogens act as partial agonists but they have no effect in the long treatment. In the vagina in the short treatment anti-estrogens provoke no significant effects, but in the long treatment they are full agonists. In neither of the two biological responses studied (growth and progesterone receptor) does tamoxifen and its derivatives block the action of estradiol. (4) The use of a monoclonal antibody to the estrogen receptor revealed quantitative differences in the activation of the estrogen receptor when bound to estradiol or tamoxifen. This observation was in agreement with the lesser extent of binding to DNA-cellulose of the tamoxifen–estrogen receptor complex as compared with the estradiol–estrogen receptor complex. This fact suggests an impaired activation of the estrogen receptor induced by tamoxifen which might be related to the different biological responses provoked by estrogens and anti-estrogens.

INTRODUCTION

Since the discovery of the first triphenylethylene-derivative with anti-estrogenic properties (MER 25, ethamoxytriphetol) [1], a wide variety of compounds with this basic structure have been synthesized. One of these, tamoxifen, was found to be the most successful in therapy, particularly for the treatment of breast cancer [2, 3].

In spite of the fact that tamoxifen can bind with high affinity to two different sites: *site A* which corresponds to the estrogen receptor and *site B* (also called AEBS, anti-estrogen binding site) [4, 5], the most accepted mechanism is that tamoxifen and other triphenylethylene derivatives carry out their biological responses through their interaction with the estrogen receptor molecule. Tamoxifen and other "anti-estrogens" can act as full estrogen agonists, partial antagonists or real agonists. These variations in the biological responses can be explained by differences in the binding affinities to the estrogen receptor, in the dissociation or association rate constants, as well as in the induction of different conformations of the receptor, as was demonstrated recently using a monoclonal antibody obtained against the estrogen receptor [6, 7].

The biological response to "anti-estrogens" is a function of: (1) the animal species; (2) the target organ; (3) the biological effect considered; (4) the experimental conditions.

Other factors could also be involved, such as metabolism, half-life, and the interaction(s) with plasma or tissular proteins or other macromolecules.

As a consequence of the extensive use of tamoxifen in cancer therapy, particular interest has been focused on its metabolism and the data show significant differences in the diverse species studied, e.g. in the human the most important metabolite is *N*-desmethyltamoxifen [8, 9], and in the rat 4-hydroxytamoxifen [10].

In a series of *in vivo* studies in this laboratory on the biological response of tamoxifen during the perinatal periods of the guinea-pig, it was shown that this compound acts as an estrogen agonist in the uterus and vagina [11, 12, 13]. We summarize here the effects of tamoxifen and its derivatives (4-hydroxytamoxifen and *N*-desmethyltamoxifen) after administration to newborn guinea-pigs, as well as in isolated uterine cells of the fetal uterus. Data of the interaction of tamoxifen with the estrogen receptor and the recognition of the tamoxifen-estrogen receptor complex by a monoclonal antibody to the estrogen receptor are also presented.

INTERACTION OF [^{3}H]TAMOXIFEN WITH THE ESTROGEN RECEPTOR. RECOGNITION OF THE ACTIVATED FORM USING A MONOCLONAL ANTIBODY

In the fetal uterus of guinea-pig, tamoxifen binds to the estrogen receptor (*site A*) with a dissociation constant (K_d) of 1.8 ± 0.4 (SD) nM and to a second binding site (*site B* or AEBS), specific for the triphenylethylene class of anti-estrogens, with a higher affinity ($K_d = 0.39 \pm 0.01$ nM) [5, 11, 14].

*To whom correspondence should be addressed.

Only the tamoxifen–estrogen receptor complex translocates to the nucleus in a "cell-free" system by a temperature-dependent process while *site B* (AEBS) does not [5].

The tamoxifen–estrogen receptor complex can be activated as determined by the increase in its binding to DNA-cellulose, by factors such as temperature, time and high salt concentrations, which activate the estradiol–estrogen receptor complex (Fig. 1) [7]. Sodium molybdate inhibits the activation of both the tamoxifen- or estradiol-estrogen receptor complexes.

In a previous study [6] we demonstrated that the monoclonal antibody D547, raised against the human estrogen receptor from MCF-7 cells [15, 16], binds selectively to the activated form of the estrogen receptor. This activated form of the receptor, which can be recognized by the antibody, was called *α form*, while the non-activated form which does not bind to the antibody was called *β form*. The estrogen receptor complexed with tamoxifen is also partially recognized by the monoclonal antibody (Fig. 2B) and this binding is induced by activating factors [7]. The correlation between the increase in the DNA-cellulose binding and the transformation of the *β form* to the *α form* suggests that the form recognized by the antibody is the activated form of the tamoxifen–estrogen receptor complex.

These observations indicate similar qualitative properties of the estrogen receptor when bound to either tamoxifen or estradiol and support the hypothesis that the action of tamoxifen is mediated by the estrogen receptor. However, the extent of activation of the estrogen receptor when bound to tamoxifen

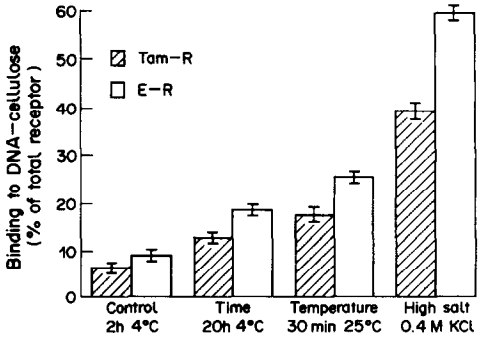

Fig. 1. Effect of activating factors on the binding of the tamoxifen- (Tam-R) and estradiol- (E-R) estrogen receptor complexes to DNA-cellulose. Aliquots of cytosol were incubated with 15 nM [^{3}H]tamoxifen or with 10 nM [^{3}H]estradiol for 2h at 4°C. Samples were treated as indicated in the figure and assayed for DNA-cellulose binding for 1 h at 4°C.

was always less than when it was bound to estradiol (Fig. 1). Similarly, the binding to the monoclonal antibody was markedly less when the receptor was complexed with tamoxifen rather than estradiol (Fig. 2A and B).

COMPARATIVE BIOLOGICAL RESPONSES OF TAMOXIFEN, 4-HYDROXYTAMOXIFEN AND N-DESMETHYLTAMOXIFEN, ALONE OR COMBINED WITH ESTRADIOL, IN THE UTERUS OF NEWBORN GUINEA-PIGS

It can be established at present that of all the animal species studied, in only two (mouse and guinea-pig) tamoxifen and other triphenylethylene derivatives act as full agonists and do not block the

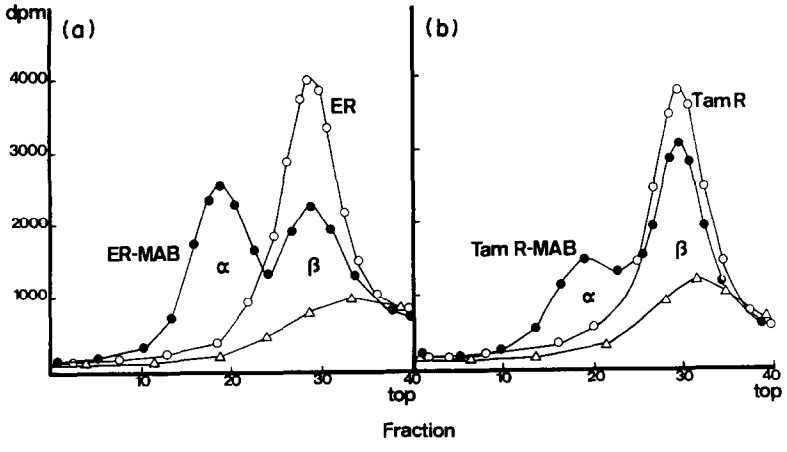

Fig. 2. Interaction of the estrogen receptor bound to estradiol (A) or to tamoxifen (B) with the monoclonal antibody. (A) Aliquots of cytosol were incubated with 10 nM [^{3}H]estradiol (-○-) or with 10 nM [^{3}H]estradiol + the monoclonal antibody D547 (-●-) for 20 h at 4°C. (B) Aliquots of cytosol were incubated with 15 nM [^{3}H]tamoxifen (-○-) or with 15 nM [^{3}H]tamoxifen + the monoclonal antibody (-●-) for 20 h at 4°C. Samples were analyzed by centrifugation through sucrose density gradients (10–30% w/v; 0.4 M KCl) at 400,000 *g* for 120 min using a Beckman vertical rotor VTi65. Non-specific binding was determined in the presence of a 100-fold excess of unlabelled estradiol (-△-). ER: estradiol–estrogen–estrogen receptor complex; *α*: *α form* of receptor (activated); *β*: *β form* of receptor (non-activated).

effect of estrogens [10, 11, 12, 17, 18]. As tamoxifen in humans is extensively metabolised, mainly into *N*-desmethyltamoxifen, and 4-hydroxytamoxifen was found as a metabolite of tamoxifen in the rat uterus, it was interesting to explore the biological responses of these two metabolites in a model in which tamoxifen is a full agonist concerning the uterotrophic effect. *N*-desmethyltamoxifen or 4-hydroxytamoxifen were injected s.c. (alone or combined with estradiol) to newborn guinea-pigs (2-days old) for a short (2 days) or a long period (12 days), and the effects on uterine growth and progesterone receptor were studied.

Uterotrophic effect

Figure 3 shows the effect of short and long treatments with *N*-desmethyltamoxifen and 4-hydroxytamoxifen on the weight of the uterus. 4-Hydroxytamoxifen provokes an effect similar to that obtained with tamoxifen. *N*-desmethyltamoxifen is also uterotrophic but significantly less intense than the other triphenyl derivatives. When administered together with estradiol, the tamoxifen derivatives do not block the estradiol effect. On the contrary, the effects are additive (Fig. 4). A significant increase in the DNA content of the uterus was also observed [19], suggesting that these tamoxifen derivatives are also involved in cell proliferation.

Progesterone receptor

Another parameter studied was the action of these derivatives on the progesterone receptor. Here the situation is different because in the short treatment there is an agonist effect greater than that provoked by estradiol, but in the long treatment the concentration of the progesterone receptor is similar to that obtained in the non-treated animals (Fig. 5). In any case the tamoxifen derivatives do not block the action of estradiol (unpublished data). This is an intriguing aspect of the anti-estrogen effect and the different possibilities and factors involved in the lack of response concerning this parameter need to be explored.

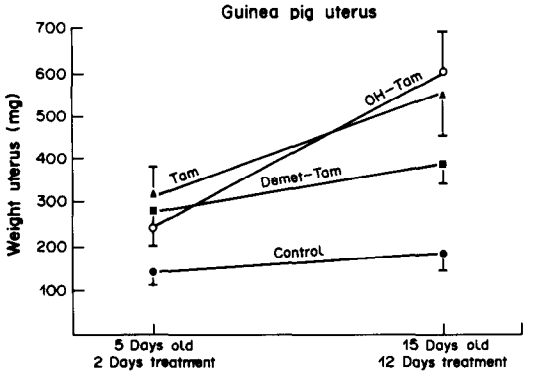

Fig. 3. Trophic effect of tamoxifen (TAM), *N*-desmethyltamoxifen (DEMET-TAM), and 4-hydroxytamoxifen (OH-TAM) in the uterus of newborn guinea-pigs treated for a short (2 days) or a long (12 days) period. Newborn guinea-pigs (2 days old) were treated subcutaneously daily for 2 or 12 days with 100 μg of each compound. The data represent the average ± SD of 6–10 animals.

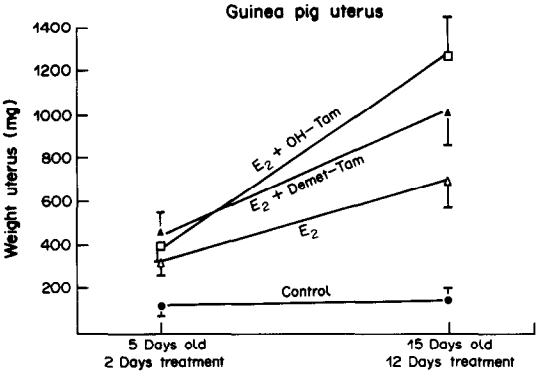

Fig. 4. Trophic effect of the combination of E_2 + OH-TAM, or E_2 + DEMET-TAM, in the uterus of newborn guinea-pigs treated for a short (2 days) or a long (12 days) period. Newborn guinea pigs (2 days old) were treated subcutaneously for 2 or 12 days with 20 μg E_2 and 100 μg of other compounds. The data represent the average ± SD of 6–10 animals.

EFFECT OF ANTI-ESTROGENS IN FETAL UTERINE CELLS IN CULTURE

Cell suspensions of fetal guinea-pig uteri were obtained by enzymatic digestion of tissue minces in 0.25% trypsin, 0.05% collagenase and 0.025% DNAse. Cells were plated in 75 cm^2 plastic flasks and cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 5% fetal calf serum. To test the effect of various added compounds, cells

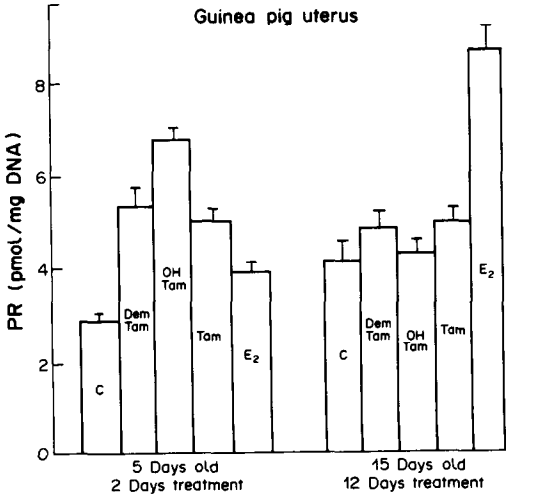

Fig. 5. Effect of tamoxifen (TAM), estradiol (E_2), desmethyltamoxifen (DEMET-TAM), and 4-hydroxytamoxifen (OH-TAM), on the progesterone receptor of the uterus of newborn guinea-pigs treated for a short (2 days) or a long (12 days) period. Legend, see Figs 3 and 4.

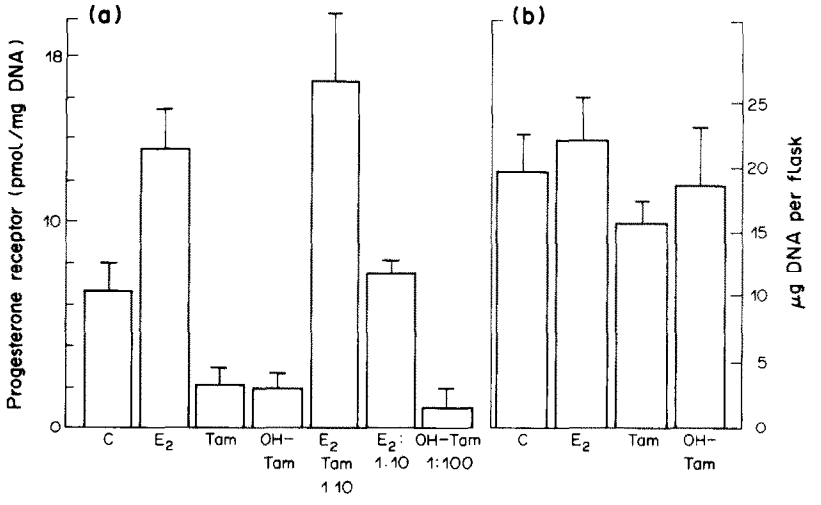

Fig. 6. Effect of anti-estrogens on progesterone receptor concentrations (A) and cell proliferation (B) in fetal uterine cells in culture. Fetal uterine cells were cultured as monolayers in plastic flasks for 9 days in DMEM + 5% charcoal-stripped fetal calf serum. Progesterone receptor concentrations were measured in the cytosol fraction and a 0.6 M KCl nuclear extract using [^{3}H]R5020 as ligand. Values are the means ±SE of 3–13 determinations. C: control with no hormone added; E_2: 1×10^{-8}M estradiol; Tam: 1×10^{-7} M tamoxifen; OH-Tam: 1×10^{-7} M 4-hydroxytamoxifen.

were replated into DMEM containing 5% charcoal-stripped fetal calf serum and cultured in the presence and absence of the test substances for 9 days [20]. As indicated in Fig. 6A, 1×10^{-8} M estradiol induces a 2-fold increase in progesterone receptor concentrations. Both tamoxifen (1×10^{-7} M) and 4-hydroxytamoxifen (1×10^{-7}) inhibited the progesterone receptor to levels well below those of cells grown in control medium. In the presence of estradiol (1×10^{-8} M), the tamoxifen (1×10^{-7} M) inhibition could be completely overcome but 4-hydroxytamoxifen was capable of effectively antagonizing the action of estradiol. It is intriguing that estrogen receptor concentrations were very low to negligible during the entire period of culture. Neither estradiol nor the anti-estrogens had any significant effect on cell proliferation (Fig. 6B).

The monolayer culture of fetal uterine cells now provides us with an *in vitro* system which is estrogen-responsive, in contrast to the spontaneous rise in progesterone receptor in the absence of estrogens previously observed in the organ culture of fetal uterine explants [21]. Although estrogen receptor concentrations decreased to very low levels compared to those found in the intact fetal guinea pig uterus, progesterone receptor could be increased by estradiol and this increase could be antagonized by anti-estrogens. Moreover, a sufficiently large excess of estradiol (10-fold) could overcome this antagonism. Whether the small quantity of estrogen receptor retains all the characteristics of the fetal uterine estrogen receptor and whether this receptor mediates the estrogen stimulation of progesterone receptor observed in the cells remains to be demonstrated, especially since the *in vitro* organ culture studies seemed to indicate the possibility of an estrogen-independent progesterone receptor. It is important to note that anti-estrogens not only inhibited the estrogen-induced increase in progesterone receptor but also decreased the concentrations as compared with cells grown in basal medium. This would suggest that the values used as the basis for the comparison of the effects of the test substances were at least partially due to intrinsic estrogenicity of the basal medium. The presence of phenol red in the medium is a possible source [22] although when tested in the fetal uterine organ culture system phenol red was not estrogenic [23].

DISCUSSION AND CONCLUSIONS

The present information shows the complexity of responses of "anti-estrogens" and confirms that the activity of these compounds is conditioned by different factors such as: the experimental conditions, the biological effects or the organ considered.

One of the interesting aspects found with the model studied is that the major metabolites of tamoxifen: 4-hydroxytamoxifen and *N*-desmethyltamoxifen can act as real estrogen agonists concerning the uterotrophic effect. In addition, in a recent work (unpublished results [19]) a significant trophic effect was also observed in the vagina. This effect was more intense with 4-hydroxytamoxifen which is known to have more anti-estrogenic effect than tamoxifen itself [24].

The question that the function of these anti-estrogens could depend on the biological response studied is raised when the effects on the progesterone receptor (PR) are considered: in the uterus of newborn guinea-pig after a short treatment period both

tamoxifen derivatives stimulated the PR with a more intense effect than estradiol itself. However, no effect was observed when the treatment was carried out for a long period.

The problem of the biological response to anti-estrogens becomes more complex when the same effect is studied in the vagina. In this organ, tamoxifen and its derivatives do not have any effect in the short treatment but the synthesis of PR is intensively stimulated after 12 days of treatment [19]. This is another intriguing aspect of the action of anti-estrogens which can have significant differences of responses in close target organs of the same species.

In addition, for these two responses (growth and PR) none of the tamoxifen derivatives studied blocked the estrogenic effect provoked by estradiol itself. It is to be remarked that in the uterus the trophic effect of the anti-estrogens can be additive to that provoked by the hormone; this was observed particularly with 4-hydroxytamoxifen.

Another important point of the anti-estrogen response is when the same biological response is compared in *in vivo* experiments or *in vitro* (organ or cell culture). Tamoxifen acts as a partial agonist in the stimulation of PR in the fetal uterus when this compound is administered to the pregnant guinea-pig. However, tamoxifen inhibits the synthesis of PR in the *in vitro* studies. The antagonistic effect is observed particularly with 4-hydroxytamoxifen, which can also block the effect provoked by estradiol, as was demonstrated in the fetal uterine cells in culture (Fig. 6B).

Tables 1 and 2 summarize the effect of tamoxifen and its derivatives in the different conditions studied.

What are the factors and/or the mechanisms which are involved in these differences of the anti-estrogen action? At the moment there is no clear reply to the phenomenon, but we can suggest that the factors (positive and/or negative) which control the different steps of "anti-estrogen" action can be completely reversed when the experimental conditions change.

Several molecular mechanisms have been proposed for the difference in intrinsic activity of estrogens and anti-estrogens, including alterations in the estrogen receptor activation [25], differences in the receptor form retained in the nucleus [26], impaired nuclear processing steps [27] and interaction with specific anti-estrogen binding sites [4, 5].

A series of studies in our laboratory showed that the extent of activation of the tamoxifen–estrogen receptor complex was always lower as compared with the estradiol–estrogen receptor complex. The data suggest that activation of the estrogen receptor by tamoxifen is partially impaired. A lower binding of the estrogen receptor to DNA-cellulose and to nuclei when it is bound to an anti-estrogen rather than estradiol has also been reported by other authors [28, 29].

Similarly, the estrogen receptor interacted less with the monoclonal antibody D547 when it was bound to tamoxifen than when it was bound to estradiol [7]. This fact may reflect either the smaller proportion of activated complexes as a result of an impaired activation of the tamoxifen–estrogen receptor complex or a different conformation of the receptor induced by tamoxifen which alters the interaction of this complex with the monoclonal antibody. This monoclonal antibody could detect no differences in the estrogen receptor from human breast tumors when complexed with estradiol or monohydroxytamoxifen [30]; however, other monoclonal [31] or polyclonal [32] antibodies were able to show differences between the estrogen receptor bound to an anti-estrogen or to an estrogen.

These observations suggest that tamoxifen and

Table 1. Effect of anti-estrogens in the fetal uterus of guinea-pig in different experimental conditions

	Treatment	Effect		PR
(I) *In vivo*	TAM	Growth + +	DNA/organ ++	+
(II) *In vitro*		DNA	[^{3}H]thymidine incorporation	
(A) Organ culture	TAM	No effect	No effect	Inhibits by 50% of control values
	4-OH-TAM	No effect	No effect	Inhibits almost completely the control values
(B) Cell culture	TAM	No effect	No effect	Inhibits almost completely the control values
	4-OH-TAM	No effect	No effect	
	TAM + E_2	No effect	No effect	E_2 overcomes the TAM inhibition
	4-OH-TAM + E_2	No effect	No effect	4-OH-TAM antagonizes E_2 stimulation

PR: progesterone receptor; TAM: tamoxifen; 4-OH-TAM: 4-hydroxytamoxifen; E_2 : estradiol. Effect: +++ very intense; ++ intense; + moderate.

Table 2. Effect of tamoxifen (TAM) and derivatives (4-hydroxytamoxifen, *N*-desmethyltamoxifen) in the uterus and vagina of newborn guinea-pigs

Organ	Treatment	Effect						
		Growth		DNA/organ		PR		$+E_2$
		Short treatment	Long treatment	Short treatment	Long treatment	Short treatment	Long treatment	Growth or PR
(I) Uterus	TAM	++	+++	++	++	++	No effect	No antagonism
	4-OH-TAM	++	+++	±	++	+++	No effect	No antagonism
	N-DEM-TAM	++	++	±	++	++	No effect	No antagonism
(II) Vagina	TAM	+	+++	No effect	++	No effect	++	No antagonism
	4-OH-TAM	++	+++	No effect	+++	No effect	++	No antagonism
	N-DEM-TAM	+	+++	No effect	+++	No effect	++	No antagonism

PR: progesterone receptor; E_2: estradiol; TAM: tamoxifen; 4-OH-TAM: 4-hydroxytamoxifen; *N*-DEM-TAM: *N*-desmethyltamoxifen. Effect: +++ very intense; ++ intense; + moderate; ± weak.

estradiol affect the estrogen receptor differently. This fact may account for the different biological responses elicited by tamoxifen and estradiol [17].

All this information, and the previous studies which showed that tamoxifen can block the stimulatory effect of estradiol in the acetylation of nuclear histones [33] or antagonize the negative effect provoked by estradiol in the cell population of the fetal thymus [34] lead to the conclusion that the mechanism of action of "anti-estrogens" is a very complex problem which needs substantial complementary information.

Acknowledgements—The expenses of this work were defrayed in part by the "Centre National de la Recherche Scientifique", France (UA No. 548) and by the "Fondation pour la Recherche Médicale Française".

REFERENCES

1. Lerner L. J., Holthaus F. J. and Thompson C. R.: A non-steroidal oestrogen antagonist (1-(*p*-2-diethylaminoethoxyphenyl) - 1 - phenyl - *z* - *p* - methoxyphenylethanol). *Endocrinology* **63** (1958) 295–318.
2. Ward H. W. C.: Antiestrogen therapy for breast cancer: a trial of tamoxifen at two dose levels. *Br. med. J.* **1** (1973) 13–14.
3. Mouridsen H., Palshof T., Patterson J. and Battersby L.: Tamoxifen in advanced breast cancer. *Cancer Treat. Rev.* **5** (1978) 131–141.
4. Sutherland R. L. and Foo M. S.: Differential binding of anti-estrogens by rat uterine and chick oviduct cytosol. *Biochem. biophys. Res. Commun.* **91** (1979) 183–191.
5. Gulino A. and Pasqualini J. R.: Specific binding and biological response of anti-estrogens in the fetal uterus of the guinea pig. *Cancer Res.* **40** (1980) 3821–3826.
6. Giambiagi N. and Pasqualini J. R.: Immunorecognition of the active form of the oestrogen receptor by using a monoclonal antibody. *Biochem. J.* **230** (1985) 203–210.
7. Giambiagi N. and Pasqualini J. R.: Activation and immunorecognition by a monoclonal antibody of the tamoxifen-estrogen receptor complex. *Biochim. biophys. Acta* **883** (1986) 559–566.
8. Fromson J. M., Pearson S. and Bramah S.: The metabolism of tamoxifen (ICI 46, 474). II. In female patients. *Xenobiotica* **3** (1973) 711–714.
9. Adam H. K., Douglas E. J. and Kemp J. O.: The metabolism of tamoxifen in humans. *Biochem. Pharmac.* **27** (1979) 145–147.
10. Borgna J. L. and Rochefort H.: Occupation '*in vivo*' des récepteurs oestrogéniques par des métabolites hydroxylés du tamoxifène. *C.r. hebd. séanc. Acad. Sci., Paris (Série D)* **289** (1979) 1141–1144.
11. Pasqualini J. R., Sumida C., Gulino A., Nguyen B.-L., Tardy J. and Gelly C.: Recent data on receptors and biological action of estrogens and antiestrogens in the fetal uterus of guinea pig. In *Progress in Cancer Research and Therapy*, Vol. 14: *Hormones and Cancer* (Edited by S. Iacobelli, R. J. B. King, H. R. Lindner and M. E. Lippman). Raven Press, New York (1980) pp. 53–64.
12. Pasqualini J. R., Nguyen B.-L., Mayrand C. and Lecerf F.: Oestrogen agonistic effects of tamoxifen in the uterus of newborn guinea pigs after short and long treatment. Biological and histological studies. *Acta endocr., Copenh.* **111** (1986) 378–386.
13. Nguyen B.-L., Giambiagi N., Mayrand C., Lecerf F. and Pasqualini J. R.: Estrogen and progesterone

receptors in the fetal and newborn vagina of guinea pig: biological, morphological, and ultrastructural responses to tamoxifen and estradiol. *Endocrinology* **119** (1986) 978–988.
14. Gulino A. and Pasqualini J. R.: Heterogeneity of binding sites for tamoxifen and tamoxifen derivatives in estrogen target and nontarget fetal organs of guinea pig. *Cancer Res.* **42** (1982) 1913–1921.
15. Greene G. L., Closs L. E., De Sombre E. R. and Jensen E. V.: Estrophilin: pro and anti. *J. steroid Biochem.* **12** (1980) 159–167.
16. Greene G. L., Nolan C., Engler J. P. and Jensen E. V.: Monoclonal antibodies to human estrogen receptor. *Proc. natn. Acad. Sci. U.S.A.* **77** (1980) 5115–5119.
17. Pasqualini J. R., Giambiagi N., Sumida C., Nguyen B.-L., Gelly C., Mayrand C. and Lecerf F.: Biological responses of tamoxifen in the fetal and newborn vagina and uterus of the guinea-pig and in the R-27 mammary cancer cell line. *J. steroid Biochem.* **24** (1986) 99–108.
18. Terenius L.: Structure-activity relationships of anti-oestrogens with regard to interaction with 17β-oestradiol in the mouse uterus and vagina. *Acta endocr., Copenh.* **66** (1971) 431–447.
19. Nguyen B.-L., Lecerf F. and Pasqualini J. R.: Biological and morphological effects of tamoxifen derivatives (4-hydroxytamoxifen, *N*-desmethyltamoxifen and *cis*-tamoxifen) in the uterus and vagina of newborn guinea-pigs. (1987) Unpublished data.
20. Sumida C. and Pasqualini J. R.: Estrogen responsiveness of fetal guinea pig uterine cells in culture. *J. steroid Biochem.* **24** (1986) 231–234.
21. Sumida C., Gelly C. and Pasqualini J. R.: De novo synthesis of progesterone receptor in the fetal uterus of guinea pig in organ culture and its control by progestins and triphenyl-ethylene antiestrogens. *Biochim. biophys. Acta* **755** (1983) 488–496.
22. Berthois Y., Katzenellenbogen J. and Katzenellenbogen B. S.: Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc. natn. Acad. Sci. U.S.A.* **83** (1986) 2496–2500.
23. Sumida C., Gelly C. and Pasqualini J. R.: Modulation of the progesterone receptor in the fetal uterus of the progesterone-primed guinea pig *in vivo* and in organ culture. *Life Sci.* (1987). In press.
24. Jordan V. C., Collins M. M., Rowsby L. and Prestwich G.: A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. *J. Endocr.* **75** (1977) 305–316.
25. Rochefort H. and Borgna J. L.: Differences between oestrogen receptor activation by oestrogen and antioestrogen. *Nature (Lond.)* **292** (1981) 257–259.
26. Eckert R. L. and Katzenellenbogen B. S.: Physical properties of estrogen receptor complexes in MCF-7 human breast cancer cells. Differences with antiestrogen and estrogen. *J. biol. Chem.* **257** (1982) 8840–8846.
27. Horwitz K. B. and McGuire W. L.: Nuclear mechanisms of estrogen action. Effects of estradiol and anti-estrogens on estrogen receptors and nuclear receptor processing. *J. biol. Chem.* **253** (1978) 8185–8191.
28. Katzenellenbogen B. S., Pavlik E. J., Robertson D. W. and Katzenellenbogen J. A.: Interaction of a high affinity anti-estrogen (α-[4-Pyrrolidinoethoxy]phenyl-4-hydroxy-α'-nitrostilbene, CI628M) with uterine estrogen receptors. *J. biol. Chem.* **256** (1981) 2908–2915.
29. Cushing C. L., Bambara R. A. and Hilf R.: Interactions of estrogen-receptor and antiestrogen-receptor complexes with nuclei *in vitro*. *Endocrinology* **116** (1985) 2419–2429.
30. Tate A. C., De Sombre E. R., Greene G. L., Jensen E. V. and Jordan V. C.: Interaction of [^{3}H]estradiol- and [^{3}H]monohydroxy-tamoxifen-estrogen receptor complexes with a monoclonal antibody. *Breast Cancer Res. Treat.* **3** (1983) 267–277.
31. Borgna J. L., Fauque J. and Rochefort H.: A monoclonal antibody to the estrogen receptor discriminates between the nonactivated and activated estrogen- and anti-estrogen-receptor complexes. *Biochemistry* **23** (1984) 2162–2168.
32. Tate A. C., Greene G. L., De Sombre E. R., Jensen E. V. and Jordan V. C.: Differences between estrogen- and antiestrogen-estrogen receptor complexes from human breast tumors identified with an antibody raised against the estrogen receptor. *Cancer Res.* **44** (1984) 1012–1018.
33. Pasqualini J. R., Cosquer-Clavreul C. and Gelly C.: Rapid modulation by progesterone and tamoxifen of estradiol effects on nuclear histone acetylation in the uterus of the fetal guinea pig. *Biochim. biophys. Acta* **739** (1983) 137–140.
34. Pasqualini J. R., Gulino A., Sumida C. and Screpanti I.: Anti-estrogens in fetal and newborn target tissues. *J. steroid Biochem.* **20** (1984) 121–128.