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High affinity binding of anti-oestrogen to the chick liver nuclear oestrogen receptor

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Tamoxifen is a potent inhibitor of specific oestrogen-induced yolk protein synthesis by chicken liver. The oestradiol receptor in salt extracts of liver nuclei from oestrogen-treated chicks has a K_D for oestradiol of 0.7 ± 0.2 nM. Tamoxifen and its metabolite, monohydroxytamoxifen, compete for binding to the salt-soluble nuclear receptor with K_i values of 2.6 and 0.1 nM respectively. The anti-oestrogens show much less inhibition of [^{3}H]oestradiol binding when assays are carried out using intact nuclei. The competition by unlabelled oestradiol for [^{3}H]oestradiol binding to receptor is identical in both salt extracts and intact nuclei. This suggests that intact nuclei contain components which bind anti-oestrogens, but not oestradiol. While tamoxifen and desmethyltamoxifen will readily dissociate from the salt-soluble nuclear oestrogen receptor, monohydroxytamoxifen does not dissociate under the conditions generally used for exchange assays. A modified assay was developed in which 60–70% of monohydroxytamoxifen-bound sites were shown to be exchangeable for [^{3}H]oestradiol. Soluble receptor preparations were first incubated in a 1.7% charcoal suspension at 37°C for 15 min before assay of specific oestradiol binding. This technique was used in examining the effects of tamoxifen and monohydroxytamoxifen given *in vivo* on the nuclear oestrogen receptor concentration. Despite their 30-fold difference in binding affinity for the receptor, both anti-oestrogens increase nuclear receptor levels to about the same degree. When given with oestradiol, both compounds have the same apparent partial inhibitory effect on the oestrogen-induced increase in nuclear receptor. These data are consistent with the metabolic hydroxylation of tamoxifen before binding to the hepatic oestrogen receptor.

Non-steroidal anti-oestrogens can be classified as compounds that inhibit the binding of oestradiol to the oestrogen receptor and, possibly as a result, prevent the full expression of oestrogenic responses in target tissues. As yet no precise molecular mechanism has been described for anti-oestrogen action although several hypotheses have been suggested. Anti-oestrogens have been observed to inhibit cytoplasmic oestrogen receptor replenishment (Clark *et al.*, 1974), to interfere with processing of nuclear oestrogen receptor (Horwitz & McGuire, 1978), and to produce some qualitative or quantitative defects in receptor activation and translocation (Rochefort & Borgna, 1981; Hayes *et al.*, 1981; Katzenellenbogen *et al.*, 1981). Overall, the structurally specific anti-oestrogen–oestrogen-receptor complex appears to have a lower intrinsic activity than the oestradiol–oestrogen-receptor complex, particularly for stimulating proliferative processes (Jordan *et al.*, 1977*a*).

Generation of a convincing general theory for the mechanism of anti-oestrogen action is frustrated by the species-specific effects of these compounds. The so-called anti-oestrogens exhibit full oestrogenic properties in short term tests in the mouse uterus (Terenius, 1971), some oestrogenic responses (e.g. progesterone receptor synthesis; Jordan & Prestwich, 1978) and anti-oestrogenic responses (e.g. inhibition of mitosis; Jordan & Dix, 1979) in the rat uterus, and apparently universal anti-oestrogenic responses in the chick oviduct (Sutherland *et al.*, 1977; Sutherland, 1981).

Tamoxifen {(*Z*)-2-[*p*-(1,2-diphenylbut-1-enyl)-phenoxy]-*NN*-dimethylethylamine}, an anti-oestrogen used in the treatment of breast cancer (Patterson, 1981), has several metabolites, monohydroxytamoxifen [(*Z*)-2-{*p*-[1-(4-hydroxyphenyl)-2-phenyl-

Abbreviation used: Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulphonic acid.

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but - 1 - enyl]phenoxy} - *NN* - dimethylethylamine] (Jordan *et al.*, 1977*b*) and desmethyltamoxifen (Adam *et al.*, 1979) that are anti-oestrogens in their own right. The metabolites may play an important role in the mechanism of action of tamoxifen because the administration of [^{3}H]tamoxifen results in the localization of monohydroxy-[^{3}H]tamoxifen in target cell nuclei (Borgna & Rochefort, 1981) and derivatives of tamoxifen that cannot undergo metabolic conversion to monohydroxytamoxifen have attenuated agonistic and antagonistic actions in the rat (Allen *et al.*, 1980).

Tamoxifen, as well as nafoxidine and CI-628, are extraordinarily potent inhibitors of specific oestrogen-induced egg yolk protein synthesis in the chick liver (Gschwendt, 1975; Lazier & Alford, 1977; Capony & Williams, 1980). However, earlier studies have shown that neither nafoxidine nor CI-628 competes to a significant degree with oestradiol binding to the liver nuclear oestrogen receptor, the presumed effector of oestrogen action (Gschwendt, 1975; Lazier & Alford, 1977). We now find that tamoxifen, and particularly its monohydroxylated derivative, exhibit high affinity binding for this receptor.

Materials and methods

Animals and injections

White leghorn cockerels of about 100g body weight were used for all experiments. Oestradiol-17β and/or anti-oestrogen was dissolved in propylene glycol/ethanol (1:1, v/v) at the concentrations indicated in the text. Injections (0.2ml/100g body weight) were given intramuscularly.

Preparations of liver fractions

Nuclei were purified from liver by centrifugation in glycerol-containing buffers using methods based on those described by Snow *et al.* (1978). Briefly, minced liver was homogenized at 0°C in 10vol. of buffer containing 10mM-Tris/HCl (pH 7.5 at 25°C)/10mM-NaCl/1.5mM-$MgCl_2$/50% (v/v) glycerol. After centrifugation at 6000*g* for 15min the crude nuclear pellet was washed twice with the homogenization buffer supplemented with detergents (0.05% Triton X-100 and 0.1% NP-40). The resulting pellet was washed twice in TGM buffer [10mM-Tris/HCl (pH 7.4 at 25°C)/1mM-$MgCl_2$/25% (v/v) glycerol] and was suspended in TGM buffer at a concentration of about 1.5mg of DNA/ml. For preparation of salt extracts, the nuclei were sedimented from suspension, and a volume of buffer B [0.5M-KCl/1.5mM-EDTA/10mM-Tes (pH 7.4)/10mM-monothioglycerol] equal to the original suspension volume was added, giving a final KCl concentration of 0.4M. After vigorous vortexing, the salt extract was frozen, thawed and centrifuged at 37000*g* for 20min. The chromatin fraction was prepared from the purified nuclei by washing three times in 10mM-Tris/HCl, pH 7.4, containing 10mM-$MgCl_2$ and 10mM-monothioglycerol, swelling for 18h in the same solution, followed by sedimentation at 10000*g* for 10min and one additional wash and final suspension in TGM buffer.

Assay of [^{3}H]oestradiol binding activity

The oestrogen receptor content of intact nuclei and of chromatin was measured by exchange at 30°C (Snow *et al.*, 1978). Incubation of nuclei or chromatin (100μl) was carried out in a total volume of 0.3 ml containing TGM buffer (100μl), TE buffer [0.01M-Tris/HCl (pH 7.5)/1.5mM-EDTA/10mM-monothioglycerol] (85μl), [^{3}H]oestradiol in TE buffer, 10μl (final concn. 8nM), and 5μl of ethanol or diethylstilboestrol (final concn. 800nM) or anti-oestrogen as indicated in the text. After incubation, washing of the nuclei or chromatin pellet was performed with TM buffer [0.01M-Tes (pH 7.5)/1mM-$MgCl_2$].

Binding activity of the nuclear salt extracts was determined by a modification of the charcoal adsorption technique reported previously (Lazier, 1978). The extracts were usually stripped of endogenous steroid by preincubation at 37°C for 15min with an equal volume of 0.5% charcoal/0.05% Dextran T70 in buffer B. After removal of the charcoal by centrifugation the extracts were incubated in a total volume of 0.3ml at 30°C for 30min with [^{3}H]oestradiol (8nM) in the absence or presence of a 100-fold excess of unlabelled diethylstilboestrol, cooled and charcoal-treated (as above) for 30 min at 0°C. Samples of the supernatant (0.4ml) were counted for radioactivity in Aquasol II (New England Nuclear) at an efficiency of about 45%.

Anti-oestrogens

Because of the photosensitivity of monohydroxytamoxifen, solutions in ethanol were freshly prepared before each experiment. The anti-oestrogens were supplied by ICI, Macclesfield, Cheshire, U.K.

Results and discussion

Binding of tamoxifen and its derivatives to the liver oestrogen receptor in vitro

Fixed concentrations of tamoxifen and monohydroxytamoxifen were incubated with nuclear salt extract and increasing concentrations of [^{3}H]oestradiol. The binding data were analysed by the classical Lineweaver–Burk method. Fig. 1 shows that both anti-oestrogens exhibit apparently competitive inhibition of [^{3}H]oestradiol binding. The K_i values for

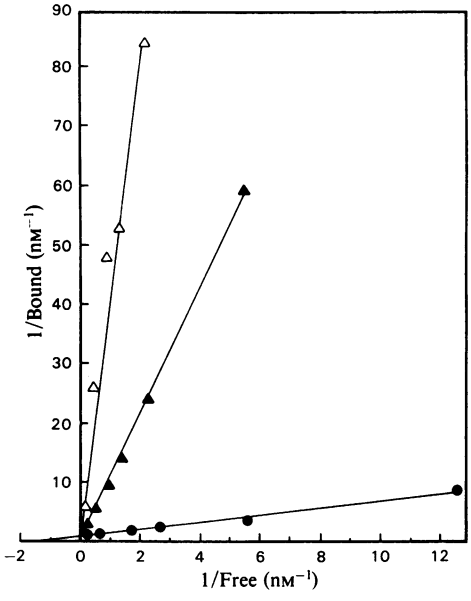

Fig. 1. *Lineweaver–Burk plot of anti-oestrogen inhibition of [^{3}H]oestradiol binding to the soluble nuclear oestrogen receptor*

Salt extract was prepared from liver nuclei of chicks which had been given oestradiol (25mg/kg) 18h earlier. Specific binding of increasing concentrations of [^{3}H]oestradiol was determined in the absence of anti-oestrogen (●) and in the presence of 25nM-tamoxifen (▲) or 2.5nM-monohydroxytamoxifen (△).

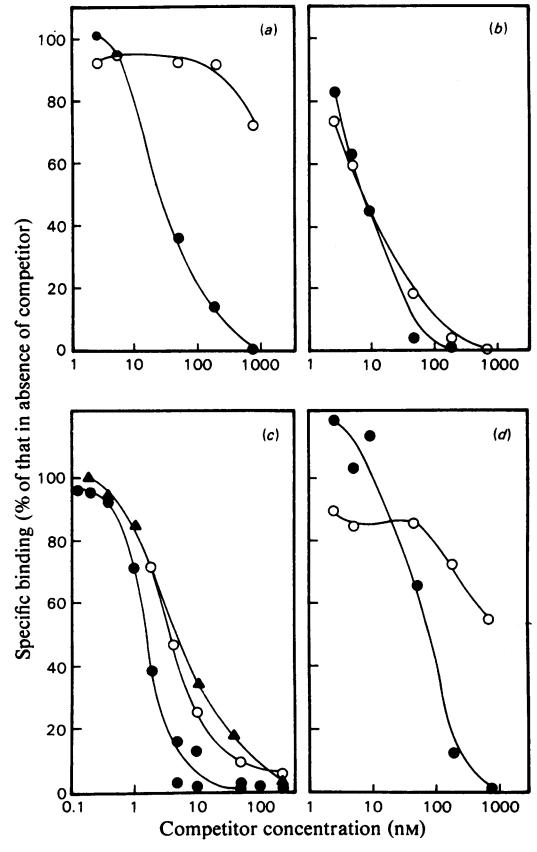

Fig. 2. *Competition of anti-oestrogens for the oestrogen receptor in intact nuclei and chromatin as compared with salt extracts*

Specific binding of 8nM-[^{3}H]oestradiol was determined in the absence or presence of increasing concentrations of anti-oestrogens in: ●, salt extracts; ○, intact nuclei; ▲, chromatin. The individual assays are detailed in the Materials and methods section. Competition was by: (*a*), desmethyltamoxifen; (*b*) oestradiol-17β; (*c*), monohydroxytamoxifen; (*d*), tamoxifen.

monohydroxytamoxifen binding are 0.10 ± 0.06nM (S.D., four preparations) and for tamoxifen are 1.5–3.6nM (two preparations). This indicates an approx. 30-fold difference in the relative affinities of the two anti-oestrogens for the receptor. The K_D for oestradiol is 0.73 ± 0.21nM (S.D., for four preparations). Thus the affinity of monohydroxytamoxifen for the oestrogen receptor is several-fold greater than that of oestradiol itself.

Competition of the anti-oestrogens for the oestrogen receptor was also assessed in assays in which a fixed, saturating, concentration of [^{3}H]oestradiol was incubated with increasing concentrations of unlabelled anti-oestrogen. In addition, we compared the competitive potential of the anti-oestrogens for receptor in intact purified nuclei and in salt extracts of the nuclei. This was of interest because studies on regulation of the nuclear oestrogen receptor had been carried out in both types of preparation (Snow *et al.*, 1978; Lazier & Haggarty, 1979).

The concentrations of unlabelled oestrogen or anti-oestrogen necessary to give 50% inhibition of [^{3}H]oestradiol binding were determined and the relative binding affinities, representing the ratio of the oestrogen concentration divided by the anti-oestrogen concentration × 100 (Fig. 2, Table 1) were determined. The concentration of unlabelled oestradiol required for 50% inhibition of binding of 8nM-[^{3}H]oestradiol was 7nM, which is close to the predicted value. Fig. 2(*b*) shows that this is the case for oestradiol competition both in nuclear salt extracts and in intact nuclei. It will be seen however that the anti-oestrogens display quite different inhibitory activity in salt extracts and in intact nuclei.

In salt extracts, a tamoxifen concentration of 79nM gives 50% inhibition, and 1.6nM-monohydroxytamoxifen produces the same effect. This

Table 1. *Relative binding affinities of anti-oestrogens and oestradiol for the oestrogen receptor in salt extracts or intact nuclei from chick liver*

Nuclei or nuclear salt extracts from liver of oestrogen-treated chicks were incubated at the indicated temperatures with 8 nM-[^{3}H]oestradiol in the presence or absence of various concentrations of competitors. Relative binding affinity is the ratio of the oestradiol concentration which gives 50% inhibition to that of the anti-oestrogen × 100. ND, not done.

	Relative binding affinity		
	Salt extracts		Nuclei
Temperature ...	0°C	30°C	30°C
Oestradiol	100	100	100
Tamoxifen	13	9	1
Monohydroxytamoxifen	360	440	140
Desmethyltamoxifen	ND	28	<1

gives a relative binding affinity for tamoxifen of 9 and for monohydroxytamoxifen of 440. The relative effectiveness of the two anti-oestrogens derived by this method is similar to that found by comparison of the specific K_i values determined on incubation for 30 min at the same temperature (30°C). The relative binding affinities of the anti-oestrogens were also determined upon incubation at 0°C for 18 h (Table 1). Unlike the case for certain other oestrogen analogues (Bouton & Raynaud, 1978), no distinct temperature-dependent differences were found.

In intact nuclei, much higher concentrations of the anti-oestrogens are required to give 50% inhibition of [^{3}H]oestradiol binding than are found for salt extracts (Fig. 2, Table 1). The relative effectiveness of monohydroxytamoxifen compared with tamoxifen is still observed, but the potency of both compounds is reduced 4–10-fold. An even more pronounced difference in inhibitory capacity is found when desmethyltamoxifen is tested in the two different receptor preparations. A possible explanation of this phenomenon is that the anti-oestrogens, but not oestradiol, incur substantial interactions with elements on the surface or on the interior of the nuclei. This results in lowering of the free inhibitor concentration available for competition with the specific oestradiol binding sites. Monohydroxytamoxifen shows less apparent affinity for oestrogen receptor in chromatin preparations than in nuclear salt extracts (Fig. 2*c*). Thus at least some of the non-receptor anti-oestrogen binding is probably intranuclear, associated with chromatin. Distinctive high-affinity anti-oestrogen binding sites which do not bind oestradiol have recently been found in cytosol from chick liver and a variety of other target organs (Sutherland *et al.*, 1980), but nuclear binding sites have not been characterized.

Table 2. *Exchange of anti-oestrogens and oestradiol bound to the soluble nuclear oestrogen receptor: conventional methods*

Salt extract from liver nuclei from oestrogen-treated chicks was prepared and charcoal-treated as described in the Materials and methods section. Samples were preincubated with 20 nM-unlabelled oestradiol or anti-oestrogen for 30 min at 30°C. After cooling, the samples were treated with charcoal (0.5% final concn.) for 15 min at 0°C, and the supernatants were assayed for specific oestradiol binding by incubation at the indicated temperature for 30 min with 8 nM-[^{3}H]oestradiol in the absence or presence of 800 nM-diethylstilboestrol.

	Specific binding (fmol/100 µl)		
Preincubation addition Temperature ...	0°C	23°C	30°C
Oestradiol-17β	2	28	44
Monohydroxytamoxifen	1	5	6
Tamoxifen	14	46	51
Desmethyltamoxifen	18	43	47
Ethanol (vehicle)	26	40	46

Exchange characteristics of the anti-oestrogens

Considering the extremely high affinity of monohydroxytamoxifen for the salt-soluble nuclear oestrogen receptor, it was imperative to demonstrate that the conditions of an exchange assay used to assess nuclear oestrogen receptor concentration after treatment *in vivo* with anti-oestrogens actually were sufficient to permit dissociation of the ligand and subsequent binding of [^{3}H]oestradiol. Furthermore, the apparent very high non-receptor binding of the anti-oestrogen in the isolated nuclei suggests that a reservoir of such ligand may be present in nuclei of anti-oestrogen-treated animals. Such ligand could dissociate during an exchange assay with [^{3}H]-oestradiol, and could inhibit binding of the labelled ligand to receptor sites and give a false impression of diminished receptor concentration. Earlier studies on the effect of anti-oestrogen treatment in chick liver or oviduct did not demonstrate that the exchange assay was thus adequate (Lazier & Alford, 1977; Binart *et al.*, 1979; Lazier *et al.*, 1981; Lebeau *et al.*, 1981).

In order to investigate exchange conditions, charcoal-treated salt extracts from liver nuclei of oestrogen-treated chickens were incubated with sufficient unlabelled anti-oestrogen or oestradiol to saturate the oestrogen receptor. The samples were treated with varying concentrations of charcoal suspension at different temperatures in order to remove unbound and possibly some, or all, of the bound ligand. The efficacy of the charcoal step was judged by comparison of [^{3}H]oestradiol binding of the presaturated charcoal-treated extracts with that

Table 3. *Exchange of anti-oestrogen and oestradiol bound to the soluble nuclear oestrogen receptor: modified methods*
Oestrogen binding sites in nuclear salt extracts were presaturated by incubation at 30°C for 30min with unlabelled oestradiol-17β, monohydroxytamoxifen or, as a control, with an equal volume (5μl) of the ethanol vehicle. Ligand concentrations were 20nM in all cases except for experiment B where 5nM-monohydroxytamoxifen was used. Following the presaturation the tubes were cooled and treated with charcoal suspension at the indicated temperature for 30min. The charcoal concentration in experiment A was 0.5% whilst for experiment B it was 1.7%. Specific binding in the charcoal-treated extracts was determined by incubation with 8nM-[^{3}H]oestradiol in the absence or presence of 800nM-diethylstilboestrol for 30min at 30°C or for 2h at 0°C followed by charcoal treatment at 0°C as described in the Materials and methods section. The data are presented as percentage of the specific binding exhibited by the ethanol-treated control preparation after the first charcoal treatment at 37°C and assay incubation at 30°C.

Experiment	Temperature of the first charcoal treatment (°C)	Assay temperature (°C)	Presaturation treatment ...	Specific binding (% of control) Monohydroxy-tamoxifen	Oestradiol	Control (vehicle only)
A	0	30		13	92	99
	30	30		23	89	104
	37	30		30	100	100
	44	30		33	80	89
B	0	0		11	18	27
	0	30		41	71	79
	30	0		24	68	85
	30	30		51	87	94
	37	0		51	111	121
	37	30		64	100	100

of the appropriate controls. Table 2 shows that the conventional charcoal treatment (0.5% charcoal for 15min at 0°C) (Sutherland & Baulieu, 1976; Lazier & Haggarty, 1979) permits quantitative detection of oestrogen receptor sites previously bound with oestradiol, tamoxifen or desmethyltamoxifen. However, sites previously bound with monohydroxytamoxifen are not detectable, possibly due to ineffective removal of all the non-receptor-bound ligand and as well to a very low dissociation rate for monohydroxytamoxifen from the oestrogen receptor. Increasing the charcoal concentration to 1.7% and the temperature of the charcoal to 37°C permits assay of the majority of sites previously bound to monohydroxytamoxifen (Table 3, A and B). The recovery is 60–70%, and is not improved by further increases in charcoal concentration, incubation temperature or time. Limited preincubation at 37°C has a stimulating effect on subsequent [^{3}H]oestradiol binding (Table 3, B); however, prolonged preincubation at 37°C in the presence of high charcoal concentrations has a deleterious effect on the receptor. Other methods of promoting the exchange of tightly bound ligands such as the use of chaotropic agents (Sica *et al.*, 1981), were not found to be useful in this system.

The effect of anti-oestrogens in vivo on nuclear oestrogen receptor

Earlier reports showed that nafoxidine, CI-628 or tamoxifen treatment of chicks resulted in an increase in the concentration of nuclear oestrogen receptor which was quantitatively considerably less than, and temporally delayed, compared with that evoked by a similar dose of oestradiol (Gschwendt, 1975; Lazier & Alford, 1977; Lazier *et al.*, 1981). In the case of tamoxifen, it is clear that this compound has a sufficient rate of dissociation from the oestradiol receptor to permit assay under the usual exchange conditions. However, if tamoxifen is metabolized to the monohydroxylated form *in vivo* and the latter compound is actually that which is bound to the oestrogen receptor, then the usual assay conditions would not be sufficient for quantitative assay. We now find that a relatively brief (4h) exposure to tamoxifen or to monohydroxytamoxifen does give rise to a 4–6-fold increase in the nuclear oestrogen receptor concentration, as assayed in salt extracts by the modified charcoal treatment procedure (Table 4). No such increase was found by using the nuclear exchange assay. The experiments *in vitro* suggest that the exchange assays with intact nuclei are not valid reflections of the actual nuclear receptor concentrations because of high non-receptor binding of anti-oestrogen. Furthermore, it is unlikely that monohydroxytamoxifen bound to the receptor would dissociate under the conditions used. The measurements of receptor concentration made by the modified charcoal treatment technique with the salt extracts more likely give a truer picture of actual receptor levels. These figures are still limited by the 60–70% efficiency of the exchange reaction for monohydroxytamoxifen. Thus, the values given for nuclear receptor concentration in monohydroxytamoxifen-treated chicks and in the tamoxi-

Table 4. *Effect of injection in vivo of anti-oestrogens on the apparent concentration of nuclear oestrogen receptor in salt extracts and in intact nuclei*

Oestradiol or anti-oestrogen was administered intramuscularly at the dose indicated. After 4h liver nuclei and salt extracts were prepared and assayed for specific [^{3}H]oestradiol binding activity. The salt extracts were charcoal-treated before assay by incubation with 1.7% charcoal suspension for 15min at 37°C. The data represent the mean ± S.D. for duplicate determinations on at least three preparations per group.

	Specific binding (fmol/μg of DNA)	
Treatment (4h)	Salt extracts	Intact nuclei
Oestradiol (3mg/kg)	0.46 ± 0.07	0.40 ± 0.12
Untreated control	0.05 ± 0.04	0.07 ± 0.02
Tamoxifen (2mg/kg)	0.22 ± 0.04	0.05 ± 0.01
Tamoxifen (6mg/kg)	0.27 ± 0.04	0.08 ± 0.02
Monohydroxytamoxifen (2mg/kg)	0.20 ± 0.03	0.05 ± 0.02
Monohydroxytamoxifen (6mg/kg)	0.24 ± 0.02	0.07 ± 0.02

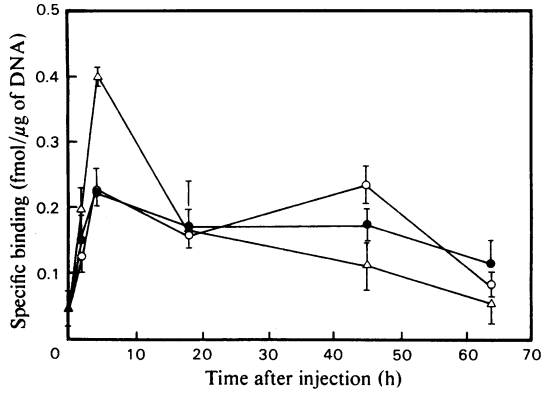

Fig. 3. *Time course of the effect of tamoxifen and monohydroxytamoxifen treatment in vivo on the soluble nuclear oestrogen receptor*

Chicks were injected with oestradiol (△, 3mg/kg) or with tamoxifen (●) or monohydroxytamoxifen (○) (each 6mg/kg). Salt extracts of liver nuclei were prepared at various times after injection and [^{3}H]oestradiol binding activity was measured in extracts which had been pretreated with 1.7% charcoal suspension at 37°C for 15min. Results are the means ± S.D. for duplicate determinations on preparations from each of three animals.

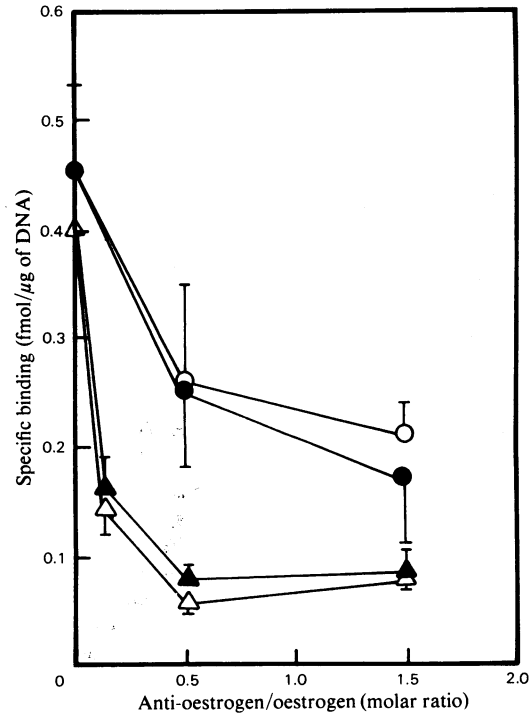

Fig. 4. *Effects of tamoxifen and monohydroxytamoxifen given with oestradiol in vivo on the apparent concentration of nuclear oestrogen receptor*

Tamoxifen (open symbols) or monohydroxytamoxifen (filled symbols) were given simultaneously with oestradiol (3 mg/kg) at the molar dose ratio indicated on the abscissa. After 4h, liver nuclei and nuclear salt extracts were prepared. [^{3}H]Oestradiol binding activity in intact nuclei was assayed by exchange at 30°C and the salt extracts were pretreated with 1.7% charcoal suspension for 15min at 37°C prior to assay at 30°C. ○,●, Salt extracts; △, ▲, intact nuclei. Results given are the means ± S.D. for duplicate determinations of preparations from each of three animals.

fen-treated chicks could be 30–40% low depending on the degree of metabolic hydroxylation.

The time course of the anti-oestrogen-induced increase in the nuclear oestrogen receptor concentration is shown in Fig. 3. Unlike in earlier experiments where higher doses and conventional exchange techniques were used (Lazier *et al.*, 1981), we now find that tamoxifen injection produces a relatively rapid and sustained effect. Similar kinetics are found for monohydroxytamoxifen, while oestradiol injection results in a peak in nuclear receptor concentration at 4h followed by gradual decline.

These results most likely reflect differential clearance rates for oestradiol and anti-oestrogens in the chicken (Binart *et al.*, 1979).

Fig. 4 shows the effect of different doses of the two anti-oestrogens given with oestradiol for 4h *in vivo* on the accumulation of nuclear oestrogen receptor. Both the nuclear exchange and the modified salt extract assays were used. Tamoxifen and monohydroxytamoxifen produce a similar degree of apparent inhibition. This is especially pronounced when binding is assessed by the assay with intact nuclei. Although the results may be artifactual in terms of true inhibition of nuclear receptor accumulation, they suggest that the metabolic transformation of tamoxifen to the higher affinity ligand is taking place.

For a mixed dose of anti-oestrogen and oestradiol the amounts of nuclear receptor-specific binding of anti-oestrogen and oestradiol would be proportional to their relative affinities for the receptor and to their intrahepatic concentrations. Thus, for a molar dose ratio of 1.5:1 (anti-oestrogen:oestrogen) it is possible that the majority of the intranuclear oestrogen receptor sites would be bound to monohydroxytamoxifen. If, as pointed out earlier, some 30–40% of these sites are not detectable in the salt extract assay, then it is obvious that there is little true inhibition of accumulation of nuclear receptor. Furthermore, the estimate that monohydroxytamoxifen constitutes the majority of nuclear-bound ligand at a molar dose ratio of 1.5:1 is consistent with observations on the striking anti-oestrogenic capacity of tamoxifen. Under these conditions, over 70% inhibition of oestradiol-induced synthesis of the apoprotein B of very low density lipoprotein is found (Capony & Williams, 1980).

Very high affinity binding of monohydroxytamoxifen relative to tamoxifen has been reported for the oestrogen receptor from chick oviduct (Binart *et al.*, 1979) and from rat and calf uterine tissues (Jordan *et al.*, 1977*b*; Borgna & Rochefort, 1980; Rochefort *et al.*, 1979). There is some controversy as to whether or not metabolism is an obligatory step in the anti-oestrogenic activity of tamoxifen. According to Binart *et al.* (1979) little monohydroxytamoxifen is found in chick oviduct after tamoxifen injection and no conversion occurs in the tissue *in vitro*, suggesting that hydroxylation is not a prerequisite. On the other hand, Borgna & Rochefort (1981) report that both oviduct and liver readily hydroxylate tamoxifen and conclude that hydroxylation is likely to be an important feature in its action. Derivatives of tamoxifen which cannot undergo hydroxylation have been tested for uterotropic effects in the rat (Allen *et al.*, 1980). Although greatly attenuated, the derivatives retain some antagonistic and agonistic potential, demonstrating that, in this case, metabolic hydroxylation is an advantage, but not an absolute prerequisite, for anti-oestrogen action. A similar situation appears to hold in the case of the human breast tumour cell line, MCF-7 (Horwitz *et al.*, 1978).

The very high affinity of monohydroxytamoxifen for the liver nuclear oestrogen receptor makes it an interesting and potentially useful compound for further study. Our results underline the necessity for thorough investigation of exchange assay conditions for anti-oestrogens and their metabolites in order to assess their effects on nuclear receptor accumulation. With one possible exception (Hayes *et al.*, 1981), it appears that reports of defective oestrogen receptor translocation by anti-oestrogens could be reinterpreted in terms of high affinity binding of the administered compound or a metabolite interfering with the nuclear exchange assay (Gschwendt, 1975; Lazier & Alford, 1977; Lazier *et al.*, 1981; Binart *et al.*, 1979; Lebeau *et al.*, 1981).

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