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Binding Properties of Zearalenone Mycotoxins to Hepatic Estrogen Receptors

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SUMMARY

POWELL-JONES, W., S. RAEFORD, AND G. W. LUCIER. Binding properties of zearalenone mycotoxins to hepatic estrogen receptors. *Mol. Pharmacol.* **20**:35–42 (1981).

This study investigates binding characteristics of three derivatives of the estrogenic mycotoxin zearalenone by components of liver cytosol. Studies show that cytosol from male and female rats contain similar amounts of specific estrogen receptors which sediment in the 8–9 S region of 5–20% sucrose gradients. In addition, male liver cytosol contains a second class of "nonreceptor" estrogen-binding sites sedimenting in the 4–5 S region of sucrose gradients. Sedimentation analyses show that each of the mycotoxin derivatives (P-1496, P-1502, and P-1560) behave in an analogous manner to the synthetic estrogen, diethylstilbestrol, in that they compete effectively for 8 S receptor sites, whereas they bind poorly to 4 S nonreceptor sites. The binding affinities of the derivatives for receptor sites were determined from competition studies at 4° by using partially purified receptors and a range of concentrations of the competing ligands. During a short (90-min) incubation period, the relative binding affinities of P-1496, P-1502, and P-1560 for receptor sites were 30, 17, and 12%, respectively, of that exhibited by 17β-estradiol. The relative binding affinity of 17β-estradiol and P-1496 did not change as a function of time at 4°. However, the relative binding affinities of P-1502 and P-1560 decreased to 6.8 and 4.2%, respectively, during an extended incubation period at 4°. The binding affinity of each derivative was similar towards partially purified liver and uterine receptors during the extended incubation period. Dissociation rate constants of 17β-estradiol, P-1496, and P-1560 were obtained indirectly by measuring the rate of exchange of the unlabeled ligand with [^{3}H]estradiol at 25°. The dissociation rate constants were 3.0×10^{-3} min^{-1}, 5.6×10^{-3} min^{-1}, and 0.14 min^{-1} for 17β-estradiol, P-1496, and P-1560, respectively. These studies show that the mycotoxin derivatives have the potential for modulating liver function through interaction with specific estrogen receptors. The true estrogen potential of the derivatives may depend upon the formation of a stable slowly dissociating ligand-receptor complex.

INTRODUCTION

The estrogen mycotoxin zearalenone is synthesized by the mold *Fusarium*, which grows commonly on major cereal crops such as corn, wheat, and maize (1). The various synthetic derivatives of zearalenone are relatively weak estrogens and their potential usefulness in a variety of areas is becoming apparent. For example, P-1496 (Fig. 1) is now being widely used as a substitute for DES[1] as a growth promotor in livestock (2). This derivative has also been tested clinically as a drug to alleviate menopausal symptoms in women (3), and some derivatives may possess clinical utility for the control of mammary tumors (4).

However, it has been known for many years that consumption of moldy corn is associated with hyperestrogenism and infertility in swine and dairy cattle (1). Moreover, zearalenone has been shown to cause fetal abnormalities (5) and has been implicated in the induction of "spontaneous" tumors of the pituitary and sex organs in animals and man (6).

One mechanism by which the mycotoxins may exert their estrogenic effects is by binding to specific cytosolic estrogen receptors in the target cell. Subsequent nuclear translocation and interaction of the receptor-mycotoxin complex with DNA may result in the modulation of RNA and protein synthesis. In spite of their nonsteroidal macrolide structure (Fig. 1), zearalenone and several of its analogues have been shown to bind to specific estrogen receptors in the rat (7) and mouse (8) uterus, in rat

[1] The abbreviations used are: DES, diethylstilbestrol; DCC, dextran-coated charcoal.

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mammary gland (9), and in human breast cancer cells (10). Translocation of the mycotoxin-receptor complex into the cell nucleus has also been demonstrated (7, 8, 10).

In recent years, the liver has come to be regarded as a target organ for estrogen. Specific estrogen receptors have been demonstrated in both cytosol and nucleus of the liver cell (11, 12). Concern has been expressed over the possible impairment of liver function in some individuals as a consequence of the interaction of synthetic estrogenic components of oral contraceptives with hepatic estrogen receptors (13, 14). It follows that the liver may also be a toxicologically important target organ for the action of environmental estrogens such as the *Fusarium* mycotoxins. The present study investigates the binding characteristics of various zearalenone derivatives to heptatic estrogen receptors and shows that dissociation kinetics may be an important determinant in assessing the true estrogenic potential of the derivatives.

MATERIALS AND METHODS

Animals

Intact male and female Sprague-Dawley rats bred at the National Institute of Environmental Health Services from a CD strain originally purchased from Charles River Breeding laboratories, Inc. (Portage, Mich.) were used throughout this study. Animals were killed by decapitation at 60–80 days of age. The animals were housed in a 12-hr dark/12-hr light environment and had free access to food and water.

Chemicals

The zearalenone derivatives P-1496, P-1560, and P-1502 (greater than 99% pure) were gifts from Dr. M. T. Shipchandler and M. Bachman of International Minerals and Chemical Corporation (Terre Haute, Ind.). 17β-[2,4,6,7-^{3}H]Estradiol (91–113 Ci/mmole; greater than 99% pure) was purchased from New England Nuclear Corporation (Boston, Mass.). Radioinert estradiol and DES were purchased from Steraloids (Wilton, N. H.). The remaining chemicals were purchased from Sigma Chemical Company (St. Louis, Mo.) and J. T. Baker Chemical Company (Phillipsburg, N. J.).

Preparation of Cytosols

Liver cytosol. Portions of liver, excised from a minimum of three animals, were pooled and placed immediately on ice. All subsequent procedures were performed at 4°. The livers were washed once in buffer A (1.0 mM dithiothreitol, 1 mM EDTA in 10 mM Tris; pH 7.4) and minced finely by using scissors. Tissue was then homogenized in five volumes of buffer A by four passes with a Teflon pestle using a Potter-Elvehjen homogenizer. Cytosol was prepared by centrifugation of the crude homogenates at 100,000 × g_{av} for 1 hr by using either the Ti-60 or the Ti-65 rotor (Beckman Instruments, Inc., Fullerton, Calif.).

Uterine cytosol. A minimum of ten uteri were pooled for each experiment. Homogenates of the minced uteri were prepared in five volumes of buffer A by using three 10-sec bursts from a Polytron PCO-2. Cytosol was prepared from the crude homogenates as described above.

Protein was quantified in liver and uterine cytosols by the method of Lowry *et al.* (15).

Competition studies using liver cytosol-sedimentation analysis

Replicate aliquots (200 μl) of liver cytosol, prepared as described above, were incubated (90 min at 4°) with 100 μl of [^{3}H]estradiol, prepared in buffer A, and either 100 μl of the competing ligand 17β-estradiol, DES, P-1496, P-1560, or P-1502), prepared in buffer A, or 100 μl of buffer A alone. The final concentration of [^{3}H]estradiol was 30 nM and the final concentration of competing ligand was 3 μM. Each incubation mixture also contained methanol (5% v/v) to aid solubility of the ligands. After the incubation period, unbound [^{3}H]estradiol was removed by treatment (30 min at 4°) with a solution (400 μl) containing 0.5% (w/v) activated, untreated charcoal powder and 0.05% (w/v) dextran (clinical grade) in buffer A (DCC solution). Charcoal was then pelleted (800 × g_{av} for 10 min) and the resulting supernatants from the replicate incubations were pooled. Aliquots (400 μl) of the supernatants were analyzed on 5–20% sucrose gradients as described previously (11).

Partial purification and quantitation of cytosolic estrogen receptors

A 60% saturated ammonium sulfate solution prepared in buffer A was added dropwise to equal volumes of liver and uterine cytosol. Mixtures were shaken on ice for 30 min and then centrifuged at 12,350 × g_{av} for 30 min. Following centrifugation, the supernatants were discarded and the precipitates, containing partially purified estrogen receptors, were resuspended in buffer A to a volume one-half that of the original cytosol.

Quantitation of estrogen receptors in uterine cytosol and in resuspended ammonium sulfate precipitates of both liver and uterine cytosol was achieved by using saturation analysis. Experimental procedures were carried out as described previously (11), except that the incubation time was reduced from 18 hr to 90 min.

Competition studies using partially purified liver receptors

Receptors were partially purified from liver cytosol as described above. Aliquots (100 μl) of resuspended ammonium sulfate precipitates were incubated (90 min at 4°) with 50 μl of [^{3}H]estradiol prepared in buffer A and either 50 μl of various concentrations of the competing ligand (17β-estradiol, P-1496, P-1560, or P-1502) prepared in buffer A or 50 μl of buffer A alone. The final concentration of [^{3}H]estradiol was 4 nM and the final concentration of ligand ranged between 1.5 and 800 nM. Each incubation mixture also contained methanol at a final concentration of 5% (v/v). After the incubation period, unbound [^{3}H]estradiol was removed by using DCC (200 μl) as described above. Aliquots of the resultant supernatants were assessed for bound radioactivity.

Determination of the relative binding affinities of the zearalenone derivatives toward liver and uterine receptors

Uterine and liver cytosol was prepared as described above. A portion of uterine cytosol was retained on ice,

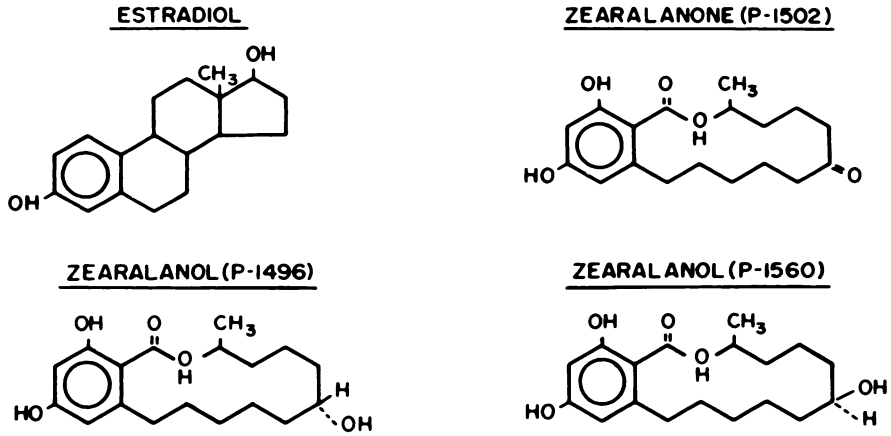

FIG. 1. *Structural formulae of the zearalenone derivatives*

during which time uterine and liver receptors were partially purified. The concentration of receptors in the subsequent resuspended ammonium sulfate precipitates from uterine and liver cytosol was quantitated by saturation analysis and volumes adjusted with buffer A in order to achieve approximately equal receptor concentrations in the three preparations. The relative binding affinity of the zearalenone derivatives toward receptors in the three preparations was then examined. The experimental procedure was identical to that outlined in the competitive binding assay described above, except that the incubation time was extended from 90 min to 18 hr.

Ligand dissociation rates

The following assay procedure was used to determine the dissociation rates of the unlabeled ligands 17β-estradiol, P-1496, and P-1560 from liver receptors, and is based on an exchange procedure described by Katzenellenbogen *et al.* (16). Receptors were partially purified from liver cytosol by using ammonium sulfate as described above and resuspended in buffer A. Portions of resuspended, partially purified receptors were incubated (90 min at 4°) with 17β-estradiol, P-1496, or P-1560 at a final ligand concentration of 50 nM. Following incubation, unbound ligand was removed by using DCC solution as described previously.

Time course of exchange with [^{3}H]estradiol. Each of the three receptor preparations saturated with the appropriate ligand were divided into two portions, to which were added (at 4°) an equal volume of 20 nM [^{3}H]estradiol (hot exchange) or 20 nM [^{3}H]estradiol plus 2 μM unlabeled 17β-estradiol (hot + cold exchange). The incubation mixtures were warmed to 25° and exchange was allowed to proceed. Periodically, aliquots were removed and the exchange of [^{3}H]estradiol was terminated by cooling to 4°. The specific binding capacity (hot exchange minus hot + cold exchange) was assayed by charcoal-dextran adsorption.

Stability determinations. Portions of resuspended, partially purified receptors were brought to 10 nM [^{3}H]-estradiol (hot preincubation) and 10 nM [^{3}H]estradiol plus 1 μM 17β-estradiol (hot + cold preincubation) and incubated (90 min at 4°) parallel to incubations with unlabeled ligands described above. During the exchange time course at 25°, the portion that was preincubated "hot" was exchanged only with 10 nM [^{3}H]estradiol and the "hot + cold" incubation only with 10 nM [^{3}H]estradiol plus 1 μM 17β-estradiol. Aliquots from these incubations give a measure of the stability of specific binding sites throughout the exchange period.

Radioactivity measurement

Sucrose gradient fractions (0.1 ml) and aliquots (0.1–0.2 ml) of incubation mixtures were measured for radioactivity in 10 ml of Ultrafluor (National Diagnostics, Inc., Somerville, N. J.) by using a Beckman LS9000 liquid scintillation counter. Counting efficiency for the samples was in the range 45–50%.

RESULTS

Competition studies using sedimentation analysis. Sedimentation analysis of [^{3}H]estradiol-labeled liver cytosol from both male and female rats revealed two peaks of protein-bound radioactivity in the 4–5 S and 8–9 S regions of gradients (Fig. 2). Previous studies in this laboratory have demonstrated that those estrogen-binding components present in the 8 S region represent specific estrogen receptors (11). Levels of 8 S binding were shown to be similar in male and female cytosol (Fig. 2). In contrast, the 4–5 S region was comprised of high capacity "nonreceptor" binding sites (11). A marked sex difference was evident in the level of those sites. Levels of 4–5 S binding were shown to be approximately 4-fold higher in male than female cytosol (Fig. 2). The presence of 100-fold excess of unlabeled 17β-estradiol in incubation mixtures containing male cytosol almost abolished [^{3}H]-estradiol binding in the 4–5 S region (Fig. 2A). On the other hand, a similar concentration of 17β-estradiol caused only minimal reduction in [^{3}H]estradiol binding to female 4–5 S components (Fig. 2B). The presence of 100-fold excess of DES had no effect on the binding of labeled estradiol to 4–5 S components in either male or female cytosol. Specific 8 S binding was completely abolished in both sexes in the presence of 100-fold excess of either 17β-estradiol or DES (Fig. 2).

Studies were undertaken to compare the competitive binding ability of the zearalenone derivatives (P-1496, P-1502, and P-1560) for liver estrogen-binding sites with that obtained for the natural and synthetic estrogens. The three derivatives totally abolished specific 8 S binding in both male and female cytosol when present in a concentration 100-fold greater than that of [^{3}H]estradiol.

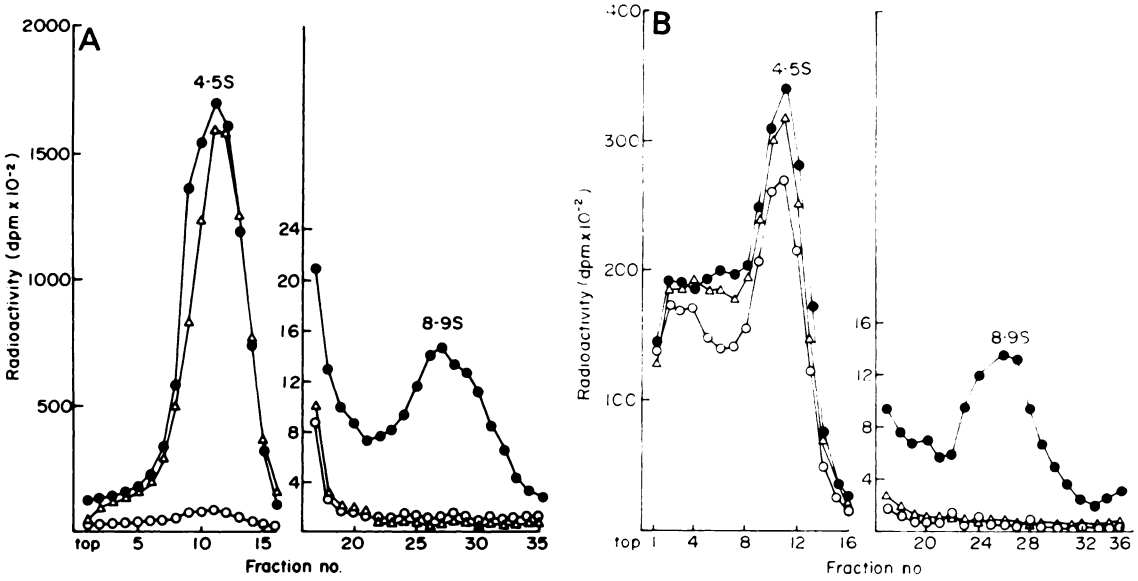

FIG. 2. *Competition by 17β-estradiol and DES for [³H]estradiol binding sites in liver cytosol*

Samples of male (A) and female (B) cytosol (protein = 10 mg/ml) were incubated (90 min at 4°) with 30 nM [³H]estradiol alone (●) or together with 100-fold excess of 17β-estradiol (○) or DES (△). Following treatment with DCC, aliquots of supernatant were analyzed on 5–20% sucrose gradients.

On the other hand, the binding of [³H]estradiol to 4–5 S components of both sexes was only minimally reduced in the presence of the derivatives (Fig. 3).

Interaction of the zearalenone derivatives with partially purified liver receptors. A more extensive investigation of the interaction of the zearalenone derivatives with liver estrogen receptors was performed by using a competitive binding assay utilizing a range of concentrations of the competing ligands. In order to eliminate complications due to [³H]estradiol binding to high-capacity 4 S components, liver receptors were partially purified by ammonium sulfate precipitation (see Materials and Methods). This procedure removes 4 S binding components. The unlabeled ligands (17β-estradiol, P-1496, P-1502, and P-1560) were then allowed to compete with [³H]estradiol for partially purified estrogen receptor sites during a relatively short (90-min) incubation period at 4°. Data presented in Fig. 4 show that 17β-estradiol competes most effectively for [³H]estradiol binding sites on the receptor. The relative binding affinities of P-1496, P-1502, and P-1560 were calculated to be 30, 17.0, and 12.0%, respectively, of that exhibited by 17β-estradiol. Similar competition curves were obtained by using either female or male partially purified liver receptors.

Comparison of the interaction of the zearalenone derivatives with liver and uterine receptors. The relative binding affinities of the zearalenone derivatives toward estrogen receptors in uterine cytosol and in ammonium sulfate precipitates of both uterine and liver cytosol were investigated by using a competitive binding assay. The assay procedure was identical to that used in the study described in the legend to Fig. 4, except that the incubation time was extended to 18 hr. Competition curves produced data illustrated in Table 1. The binding affinities of the zearalenone derivatives toward receptors were calculated relative to the affinity exhibited by 17β-estradiol. Values presented in Table 1 showed that in each of the three preparations, the relative binding affinities of the derivatives are in the order P-1496 > P-1502 > P-1560. It is also evident from Table 1 that the derivatives exhibit similar relative binding affinities toward partially purified liver and uterine receptors. However, binding of zearalenone derivatives to uterine receptors is increased by partial purification. In contrast, the binding of 17β-estradiol is similar in each of the unpurified or purified preparations from liver or uterus.

Ligand dissociation rates. The binding affinities of 17β-estradiol and the zearalenone derivatives toward partially purified liver receptors during short (90-min) and extended (18-hr) incubation periods were compared. Data presented in Table 2 are derived from the results of the competition studies performed as described in Fig. 4 and Table 1. The relative binding affinity of P-1496 did not change as a function of time. However, the relative binding affinity of both P-1502 and P-1560 decreased markedly during the extended incubation period at 4°. These data suggested that the dissociation rate of P-1496 and 17β-estradiol were similar to and slower than that of P-1502 and P-1560. A more extensive determination of the dissociation rates of 17β-estradiol, P-1496, and P-1560 was carried out. Dissociation rate constants were obtained indirectly by measuring the rate of exchange of unlabeled ligand during a 100-min incubation at 25°. Representative binding data are shown in Fig. 5A. Dissociation rates of the ligands are in the order P-1560 > P1496 > 17β-estradiol. Stability determinations demonstrated no decrease in the binding capacity of the receptor at either 4 or 25°. Dissociation rate constants of the ligands were calculated from the semilogarithmic plot of the binding data (Fig. 5B) and are tabulated in Table 3.

DISCUSSION

Previous studies in this laboratory have demonstrated that cytosol, prepared from the liver of male and female

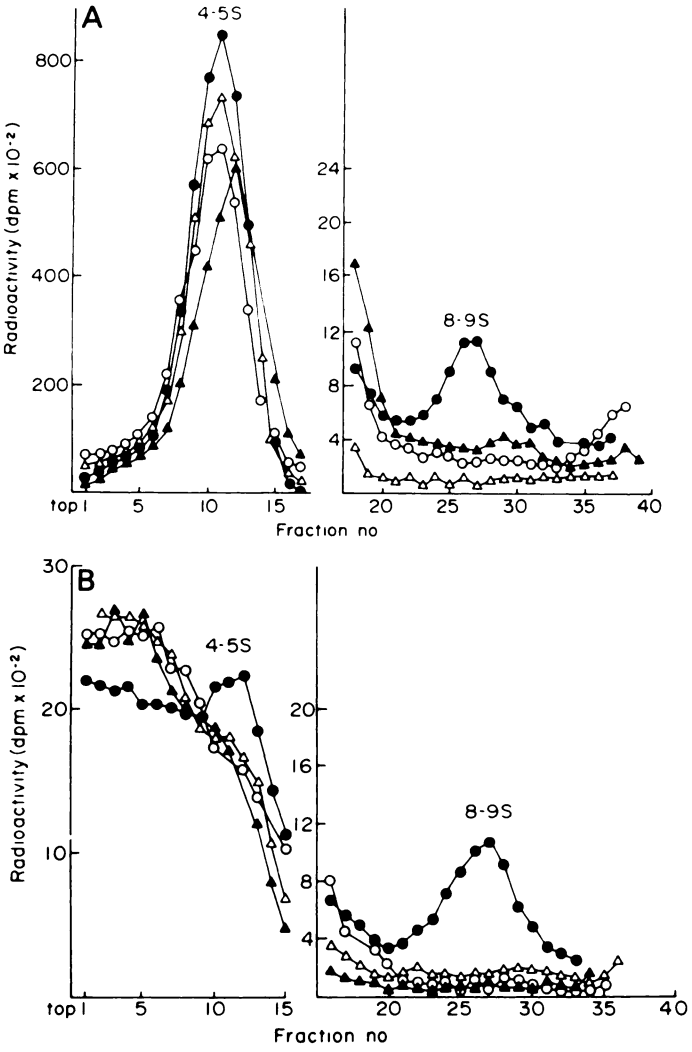

FIG. 3. *Competition by the zearalenone derivatives for [^{3}H]estradiol binding sites in liver cytosol*

Samples of male (A) and female (B) cytosol (protein = 10 mg/ml) were incubated (90 min at 4°) with 30 nM [^{3}H]estradiol alone (●) or together with 100-fold excess of P-1496 (○), P-1502 (△), or P-1560 (▲). Following treatment with DCC, aliquots of supernatant were analyzed on 5–20% sucrose gradients.

rats, contains similar amounts of specific 8 S estrogen receptors. However, male liver cytosol contains another estrogen-binding component which is distinct from the estrogen receptor. This second component, which sediments in the 4–5 S region of sucrose gradients, is apparently lacking or present in lower amounts in female liver cytosol (11). The sex difference observed (Fig. 2) with respect to the competitive binding ability of 17β-estradiol to 4–5 S components is consistent with previous observations made in this laboratory (17) which have indicated that qualitative differences exist between the sexes in the type of estrogen-binding species sedimenting in that region of sucrose gradients. The three mycotoxins (P-1496, P-1502, and P-1560) interact with estrogen-binding proteins in a manner analagous to DES, in that they compete poorly with [^{3}H]estradiol for 4–5 S binding sites (Fig. 3). The physiological significance of this finding remains unclear at the present time, since the role of 4–5 S binding components in hepatic hormone action remains to be delineated. *In vitro* studies suggest that this second class of estrogen-binding sites may act as a sequestering mechanism potentiating the biological effectiveness of endogenous estrogens by concentrating them in the target cell and facilitating nuclear translocation (11).

Results from sucrose gradient studies (Figs. 2 and 3) show that, despite their nonsteroidal macrolide nature, the three mycotoxins compete effectively with [^{3}H]estradiol for specific 8 S receptor sites in either male or female cytosol. It has been demonstrated in the mammary gland that the parent compound zearalenone inhibits the binding of [^{3}H]estradiol at the receptor site in a competitive manner (9). A possible molecular basis for the mycotoxin-receptor interaction has been postulated which assumes folding of the toxin molecule such that hydroxyl or potential hydroxyl groups become appropriately orientated to facilitate binding to unoccupied receptor sites (18).

Results from a more extensive competitive binding study (Fig. 4) utilizing a range of concentrations of the mycotoxins shows the relative binding affinity of the derivatives to be in the order P-1496 > P-1502 > P-1560. These data are in agreement with results from a previous study (4) which showed P-1496 to compete better than

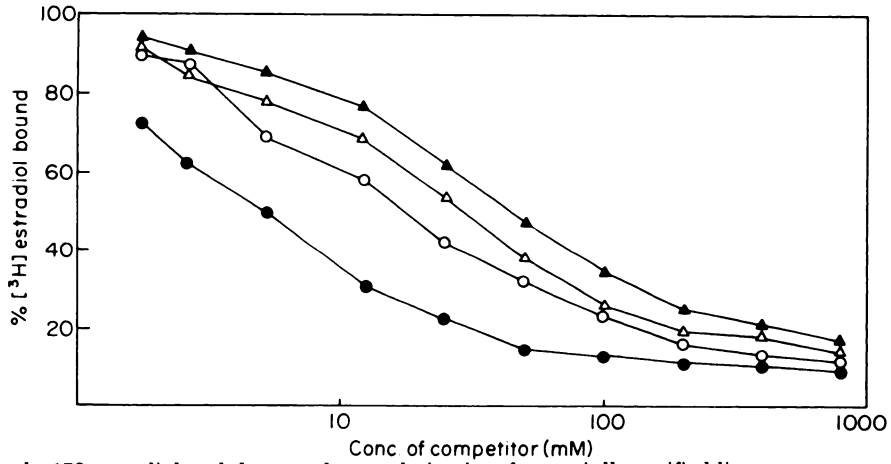

FIG. 4. *Competition by 17β-estradiol and the zearalenone derivatives for partially purified liver receptors*

Samples of redissolved ammonium sulfate precipitates of female liver cytosol were incubated (90 min at 4°) with a constant concentration of [^{3}H]estradiol (4 nM) and various concentrations (1–800 nM) of 17β-estradiol (●), P-1496 (○), P-1502 (△), or P-1560 (▲). Following treatment with DCC, aliquots of supernatants were assessed for bound radioactivity. Each value represents the average of at least two determinations.

TABLE 1

Relative binding affinities of the zearalenone derivatives toward liver and uterine estrogen receptors

Values represent the average of two separate determinations. Samples of uterine cytosol or of redissolved ammonium sulfate precipitates of uterine or liver cytosol were incubated (18 hr at 4°) with constant concentrations of [^{3}H]estradiol (4 nM) and various concentrations (1–800 nM) of 17β-estradiol, P-1496, P-1502, or P-1560. Following treatment with DCC, aliquots of supernatants were assessed for bound radioactivity.

$$\text{Relative binding affinity} = \frac{IC_{50}\ \text{estradiol}}{IC_{50}\ \text{derivative}} \times 100$$

IC_{50} = the concentration of unlabeled ligand required to displace or inhibit [^{3}H]estradiol binding by 50%.

Derivative	Relative binding affinity		
	Uterine receptor	Partially purified uterine receptor	Partially purified liver receptor
		%	
P-1496	9.3	28.9	25.0
P-1502	3.8	11.8	8.0
P-1560	0.76	2.5	2.9

P-1560 for specific estrogen receptors in the rat uterus (P-1502 was not used in that study). The relative binding affinities of P-1496 and P-1560 for uterine receptors are reported as being 13.6 and 0.8%, respectively, of that exhibited by 17β-estradiol, whereas our data using liver estrogen receptors (Fig. 4) yields relative binding values of 30% for P-1496 and 12% for P-1560. Due to the large amount of nonspecific binding components in liver cytosol, it was necessary to partially purify hepatic receptors prior to the competitive binding assay. Since unpurified receptors present in whole cytosol fractions were used in the uterine study, subsequent experiments were designed to more closely compare the interaction of the mycotoxins with liver and uterine receptors. Data presented in Table 1 show that following ammonium sulfate precipitation of liver and uterine cytosol, the mycotoxins exhibit almost identical relative binding affinities towards the subsequent partially purified hepatic and uterine receptors, whereas an approximately 3-fold lower affinity is exhibited by each of the mycotoxins towards unpurified uterine receptors present in whole cytosol fractions. This difference may have resulted from a decrease in the effective concentrations of the mycotoxins in whole cytosol due to (a) metabolism of the derivatives by uterine cytosolic enzymes or (b) interaction of the derivatives with cytosolic nonspecific estrogen-binding components.

An interesting finding arising from our study was that the competitive binding ability of P-1560 and P-1502 was dependent upon the length of the incubation period of

TABLE 2

Time dependency of the competitive binding ability of the zearalenone derivatives to liver receptors at 4°

Values represent the average of two determinations and were obtained from competition studies similar to those described in Fig. 4 and Table 1. Relative binding affinities were calculated as described in Table 1.

Competing ligand	Relative binding affinity	
	90-min incubation	18 hr incubation
	%	
P-1496	30.0	30.0
P-1502	17.0	6.8
P-1560	12.0	4.2

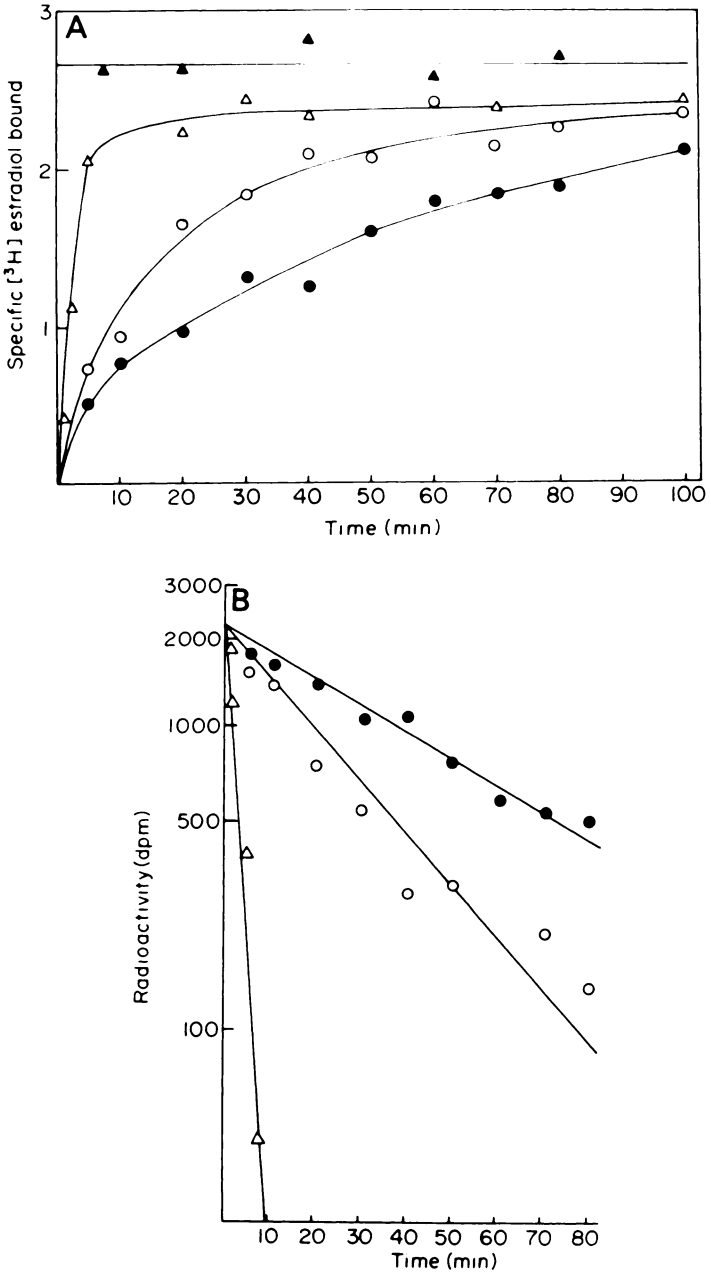

FIG. 5. *Exchange of unlabeled ligands with [^{3}H]estradiol at 25°*

A. Partially purified liver receptors were presaturated at 4° with either 17β-estradiol (●), P-1496 (○), or P-1560 (△) at final concentrations of 50 nM. Following DCC treatment, exchange of the unlabeled ligands with [^{3}H]estradiol (10 nM) was allowed to proceed at 25° for varying lengths of time. Stability of the [^{3}H]estradiol-receptor complex (▲) was determined in parallel incubations.

B. The amount of specifically bound [^{3}H]estradiol at each time point was subtracted from the value obtained for 100% exchange = 2400 dpm. The similogarithmic plot of the data is presented in B. Each value represents the average of at least two determinations.

Experimental protocol is described in detail under Materials and Methods.

TABLE 3

Ligand dissociation rates

Values represent the average of two determinations and were obtained from data presented in Fig. 5B. The half-time of exchange corresponds to the time taken to achieve 50% exchange of unlabeled ligand with [^{3}H]estradiol.

Ligand	Half-time of exchange	Dissociation rate constant at 25°
	min	
E_2	228	3.0×10^{-3} min^{-1}
1496	123	5.6×10^{-3} min^{-1}
1560	5	0.14 min^{-1}

the competitive binding assay at 4° (Table 2). Since our initial experiment (Fig. 4) was carried out over a relatively short (90-min) incubation period, whereas an extended (18-hr) incubation was used in the uterine studies, it follows that the observed differences in the relative binding affinity of P-1560 towards liver and uterine receptors may also, in part, be explained by the time-dependency of the receptor-P-1560 interaction. The time-dependent decrease in the relative binding affinity of P-1502 and P-1560 indicates that dissociation of those ligands occurs during extended incubation periods allowing for association of [^{3}H]estradiol with the subsequent unoccupied binding sites. An alternative explanation would be that differences in metabolism of β-estradiol, P-1496, P-1502, and P-1560 under the specified incubation conditions produce apparent differences in dissociation kinetics.

A more extensive investigation of ligand dissociation rates from the liver estrogen receptor was undertaken. Recent studies by Weichman and Notides (19) have shown that dissociation of 17β-estradiol from the uterine receptor occurs in two exponential phases, the faster phase from the nonactivated state of the receptor (k_{-1} = 0.12 min^{-1}) and the slower phase from the activated state of the receptor ($k_{-2} = 4 \times 10^{-3}$ min^{-1}). This biphasic characteristic of the dissociation rate of 17β-estradiol from the uterine receptor has been noted by others (4). In contrast, our data (Fig. 5B) shows that 17β-estradiol dissociation from the liver receptor occurs as a single exponential process with a dissociation rate of 3.0×10^{-3} min^{-1}, a value corresponding to the k_{-2} rate demonstrated by Weichman and Notides (19) for the activated uterine receptor. The presence of a nonactivated faster dissociating form of the liver receptor may have been detected if more time points had been employed during the first 30 min of the incubation period. Alternatively, there is evidence in the literature (20) to indicate that use of ammonium sulfate in the partial purification procedure may have increased the fractional activation of the receptor prior to the exchange process. Both P-1496 and P-1560 also dissociate from the liver receptor in a single exponential process. The rate of dissociation of P-1496 at 25° is approximately one-half that of estradiol ($k_{-1} = 5.6 \times 10^{-3}$ min^{-1}). P-1560, on the other hand, dissociates at an approximately 50-fold faster rate than 17β-estradiol (k_{-1} = 0.14 min^{-1}). It is not known whether this rapid dissociation rate represents dissociation from activated or nonactivated states of the receptor.

Studies performed in the uterus have shown that more slowly dissociating ligands have a greater capacity to translocate and maintain receptors in the nucleus, and consequently are more effective in promoting and maintaining long-term estrogenic effects than are faster dissociating ligands (21). Our results indicate that P-1496, although less effective than estradiol, would be more effective than P-1560 in maintaining long-term estrogenic effects. In this regard, it has been demonstrated that P-1496 is more effective than P-1560 in stimulating uterine weight in a 3-day uterotropic assay (4). Although differences in pharmacokinetic parameters of the mycotoxin derivatives may play a role, our data suggest that the relatively weak estrogenicity of P-1560 compared with that exhibited by P-1496 may reflect its inability to form a stable, i.e., slowly dissociable receptor complex.

In summary, our studies have shown that various mycotoxin derivatives have the potential for modifying liver function through interaction with specific estrogen receptors. Marked differences are observed *in vitro* in the duration of receptor occupancy of the drivatives. These differences may be particularly relevant when assessing the true estrogenic impact of the mycotoxins on target organ function as a result of either pharmaceutical administration or environmental exposure.

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