ELSEVIER

The Science of the Total Environment 225 (1999) 49–57

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

Amphibians as a model to study endocrine disruptors: I. Environmental pollution and estrogen receptor binding

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Abstract

Many chemicals released into the environment without toxicological risks have the capacities to disrupt the function of endocrine systems. These endocrine disruptors disturb normal endocrine mechanisms and have been observed in nearly all classes of vertebrates. The aim of this research is to develop a comprehensive model to study endocrine disruption using the amphibian *Xenopus laevis*. The assessment of estrogenic potencies of endocrine disruptors includes several levels of investigation: (I) binding to liver estrogen receptor, (II) estrogenic activity in vitro by inducing vitellogenin synthesis in primary cultured hepatocytes, and (III) in vivo effects on sexual development caused by exposure of larvae. The present paper is focused on the first part by establishing a radioreceptorassay for [^{3}H]17β-estradiol ([^{3}H]E2) binding using liver cytosol fraction. In order to get optimum binding conditions we performed kinetic, saturation, and competitive displacement experiments. Association of [^{3}H]E2 to estrogen receptor revealed that maximum specific binding is achieved between 18 and 48 h of incubation. Scatchard analyses of saturation experiments resulted in a homogenous saturable population of estrogen receptors having no significant differences of binding parameters between both sexes. The values of K_d (dissociation constant) in males and females were 22.4 $\pm$ 6.0 and 15.0 $\pm$ 2.8 nM (mean $\pm$ S.E.M.; $n = 5$), respectively, while corresponding B_{max} (maximum binding capacity) revealed 89 $\pm$ 46 and 136 $\pm$ 46 fmol [^{3}H]E2/mg protein. The specificity of estrogen receptors as shown by competitive displacement experiments demonstrated receptors being highly specific just for estrogens, but not for other endogenous steroids having the following ranking of binding affinities: E2 > estrone > dehydroepiandrosterone > aldosterone $\geq$ testosterone $\geq$ corticosterone $\geq$ progesterone. The affinity ranking of environmental chemicals compared to E2 was: E2 > tetrachlorbiphenyl > diethylphthalate > 2,2-bis-(4-hydroxyphenyl)-propan (bisphenol A) $\geq$ 4-nonylphenol $\geq$ 3-*t*-butyl-4-hydroxyanisole $\geq$ 4-octylphenol > dichlor-diphenyl-trichlor-ethan (4,4′-DDT). Analyses of five sewage effluents for displacement of [^{3}H]E2 binding resulted in three samples displacing more than 50% of specific binding at their original concentration. Taken together the established radioreceptorassay for [^{3}H]E2 binding in *Xenopus laevis* liver cytosol is useful to screen estrogen receptor binding of pure compounds or complex mixtures of them, which is the prerequisite for causing either estrogenic or antiestrogenic effects. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Amphibians; Endocrine disruptors; Estrogen receptor binding

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PII:S0048-9697(98)00331-3

1. Introduction

Recently, several studies showed that chemicals released into the environment without direct toxicological risks have capacities to disrupt development and function of endocrine systems of wildlife and humans (Colborn and Clement, 1992). These so-called 'endocrine disruptors' disturb normal endocrine feedback mechanisms either by mimicking a hormone (Pelissero et al., 1991) or by blocking humoral effects. Such compounds are suspected to contribute to the increasing rate of breast cancer (Davis et al., 1993), testicular cancer, hyperspadism, and a progressive fall in sperm count (Sharpe and Skakkebaek, 1993). Natural and synthetic chemicals may have disrupting effects acting as estrogens, antiestrogens, antiandrogens, and thyroactive agents (Colborn et al., 1993), but most observations that have been made deal with the 'feminization' phenomenon that may be caused mainly through estrogenic effects of environmental chemicals in nearly all classes of vertebrates (Schäfer et al., 1996). Although these endocrine disrupting chemicals have a quite heterogenous nature all of them are lipophilic, just like the natural occurring steroid and thyroid hormones. Demasculinization and feminization can be observed in fish (Munkittrick et al., 1991; Pelissero et al., 1993), reptiles (Guilette and Crain, 1996), birds (Fry and Toone, 1981), and mammals (Beland, 1989) derived from areas where the presence of numerous anthropogenic chemicals such as fungicides, herbicides, pesticides, and industrial effluents (Colborn et al., 1996) is evident. However, despite the dramatic problem of the declining amphibian populations all over the world (Blaustein, 1994; Stebbins and Cohen, 1995) only a few studies have paid attention to a possible contribution of endocrine disruptors for the disappearance of amphibians.

In order to obtain a better insight of the possible threat of environmental chemicals to wildlife, especially to amphibians, we are developing a model using amphibians to study endocrine disruptors. The main focus of our present research is the detection of estrogenic potencies of chemicals as well as of samples taken from the environment. The pre-requisite for estrogenic effects of a compound is its binding to cytosolic estrogen receptors in a target cell followed by dimerization of the ligand–receptor-complex. The dimer binds to DNA and promotes an estrogen-specific gene expression pattern (Beato et al., 1995; Nimrod and Benson, 1996). One of the major target organs for estrogens is the liver where in all egg laying vertebrates the induction of vitellogenin is driven specifically by the female sexual steroid 17β-estradiol (E2) (Perlman et al., 1984; Tata, 1987). Furthermore larval sexual development of amphibians is hormone dependent meaning that despite a genotypic distribution of 50% males and 50% females treatment by androgens or estrogens may shift sexes of the breed to a phenotypic complete masculinization or feminization, respectively (Witschi, 1971).

Therefore, the biology of amphibians makes them a very good tool to assess estrogenic potencies of environmental chemicals and samples including several levels of investigation: (I) binding to liver estrogen receptor, (II) biological significance in vitro by monitoring synthesis of the estrogenic biomarker vitellogenin in primary cultured hepatocytes, and (III) in vivo effects on intact animals by determination of sexual differentiation during larval development.

The present study focuses on establishing a radioreceptorassay of estrogen receptors using cytosolic liver homogenates of *Xenopus laevis* in order to get a useful tool for investigating estrogen receptor binding of pure compounds as well as of samples taken from the environment.

2. Materials and methods

2.1. Animals

Adult *Xenopus laevis* of both sexes weighing between 30 and 50 g (about 2 years old) were raised from the animal stock of the Department of Zoology II, University of Karlsruhe. They were kept in tanks containing tap water under constant environmental conditions, with a 12-h light (06:00–18:00 h) and 12-h dark (18:00–06:00 h) cycle, at a temperature between 18°C and 21°C. Animals were fed twice a week with pellets containing about 40% protein.

2.2. Materials

[^{3}H]17β-Estradiol (specific activity: 50–52 Ci/mmol) was purchased from Amersham Buchler (Braunschweig). Incubation buffer (pH 7.4) contained 20 mM Tris–HCl, 250 mM sucrose, 10 mM sodium molybdate, and 5 mM dithiothreitol (all Merck, Darmstadt). Activated charcoal solution was set up with 20 mM Tris–HCl buffer (pH 7.4) by adding 3.75 g activated charcoal Norit A (4–7 μm) (Serva, Heidelberg) and 0.375 g Dextran T70 (Roth, Karlsruhe). Unlabeled steroids such as 17β-estradiol (E2), estrone, aldosterone (Aldo), corticosterone (B), dehydroepiandrosterone (DHA), progesterone (P4), and testosterone (T) as well as the chemical 3-*t*-butyl-4-hydroxyanisole (HA) were obtained from Sigma (Deisenhofen) while the chemicals 4-nonylphenol (NP), 4-octylphenol (OP), 2,2-bis-(4-hydroxyphenyl)-propan (bisphenol A) and diethylphthalate (DEP) were purchased from Sigma-Aldrich (Steinheim) and the source of dichlor-diphenyl-trichlor-ethan (4,4′-DDT) and tetrachlorbiphenyl (TCB) was Riedel-de-Haen (Seelze). [^{3}H]17β-Estradiol ([^{3}H]E2) was dissolved in 5% ethanol while all unlabeled ligand solutions were prepared with 96% ethanol (Merck, Darmstadt).

2.3. Methods

2.3.1. Binding experiments

Preparation of cytosol. Animals were sacrificed by transsection and pithing of the spinal cord. The liver was excised rapidly, transferred into ice-cold incubation buffer, cut into small pieces of about 1 mm^3, and rinsed twice in chilled buffer to get rid of blood contents. The tissue was homogenized in five volumes of the buffer by six strokes at 1000 rev./min in a Potter tissue grinder with a motor-driven Teflon pestle (Braun, Melsungen). The homogenate was centrifuged at 12 000 $\times g$ at 4°C for 10 min, the supernatant without the lipid layer above was subjected to a second centrifugation at 100 000 $\times g$ at 4°C for 60 min, and the final supernatant containing cytosol including cytosolic steroid receptors in incubation buffer was used for binding experiments. Protein content of cytosolic fraction was determined following Lowry et al. (1951).

Binding studies include kinetic (association), saturation, and competitive displacement experiments. In preliminary experiments we tested several incubation buffers and temperatures to precheck optimal binding conditions. Best results were received clearly with the buffer described above in combination with the incubation temperature of 4°C. In all experiments incubations were performed using 25 μl [^{3}H]E2, 10 μl unlabeled ligand or solvent (96% ethanol), 150 μl incubation buffer and 100 μl cytosol prepared as described above. Separation of receptor bound and free ligands was accomplished by using dextran-coated activated charcoal solution. Three hundred μl of charcoal solution were added to each sample, vortexed, and centrifuged at 3500 $\times g$ after 5 min for 15 min at 4°C. Free ligands were absorbed by activated charcoal and sedimented while macromolecular e.g. receptor bound was present in supernatant. Therefore, receptor bound [^{3}H]E2 could be measured by taking a 300-μl aliquot of supernatant into a scintillation vial containing 3 ml scintillation cocktail (Ultima Gold; Canberra-Packard, Frankfurt) and counting in a liquid scintillation counter (Tri Carb 2000; Canberra-Packard). Each sample was performed in triplicate.

2.3.2. Kinetic experiments

Association of [^{3}H]E2 binding to estrogen receptors was repeated three times in order to get optimal incubation conditions for maximal binding for female and male liver cytosol in parallel. The time course of total binding (TB) was assayed at 1, 3, 6, 9, 12, 18, 24, 30, 48, and 72 h at a final concentration of 13.6 nM [^{3}H]E2, while nonspecific binding (NB) was determined in parallel at the same [^{3}H]E2 concentration in the presence of an excess of unlabeled E2 (10^{-5} M).

2.3.3. Saturation experiments

Saturation binding studies were carried out using liver cytosol of five male and five female individuals in parallel for 24 h incubation. Final concentrations of [^{3}H]E2 used for TB were 0.9,

1.0, 1.8, 3.1, 5.9, 11.4, 22.6, 43.1, and 89.8 nM, respectively. NB was accomplished in parallel but in the presence of a 100-fold excess of unlabeled E2.

2.3.4. Competitive displacement experiments

Displacements were performed in the absence or the presence of endogenous unlabeled ligands such as E2, estrone, Aldo, B, DHA, P4, and T at final concentrations ranging from 10^{-9} to 10^{-3} M. Cytosolic fractions were prepared from four individuals of males and females, respectively, and in all experimental set-ups [^{3}H]E2 was used at a concentration of 14.2 nM. Competitive displacements by exogenous ligands were assayed using three males and three females with 18.9 nM [^{3}H]E2 in the presence of HA, NP, OP, bisphenol A (BA), DEP, DDT, and TCB in comparison with E2. All unlabeled ligands were added at concentrations ranging from 10^{-9} to 10^{-3} M except DEP (from 0.75×10^{-9} to 2.5×10^{-4} M) and TCB (from 10^{-9} to 10^{-6} M).

In order to detect any competitive displacing effects for estrogen receptor binding of sewage effluents we modified our experimental protocol. The final experimental set up was similar as described above except minor changes. Cytosol preparation was performed using only one volume incubation buffer instead of five volumes and thus only 25 μl cytosol preparation were used for the experimental set up containing 25 μl [^{3}H]E2, 10 μl 96% ethanol with (NB) or without (TB) 10^{-5} M E2, and 225 μl incubation buffer. Incubation buffer was dissolved in artificial tap water (same osmolarity like sewage effluents (40 mosmol)) accomplished by adding artificial sea salt (aquamarin, Dreieich) for the determination of TB and NB or prepared with several dilutions of sewage effluent. Five samples of sewage effluents (obtained from Dr Jürgen Zipperle, LFU, Karlsruhe) with no genotoxicological risks were sterile filtered and kept frozen at -20°C until use. Sewage dilutions were done by diluting original samples with artificial tap water to get final concentrations of 1:1, 1:5, 1:10, and 1:50. Incubation was carried out with 17.3 nM [^{3}H]E2 over 24 h and measurement of receptor bound [^{3}H]E2 was accomplished as described above.

Analyses of saturation experiments for the binding parameters B_{max} (maximum binding capacity) and K_d (dissociation constant) were performed by Scatchard-plot analyses (Scatchard, 1949) and IC_{50}-values (concentration where 50% of specific binding is displaced) were determined from competitive displacement experiments by transforming the displacement curve in a logit–log-plot and calculating the intersection of the linear regression and the x-axis.

In order to evaluate statistical significance, the Kruskal–Wallis test was used and combined with the Mann–Whitney U-test.

3. Results

3.1. Kinetic experiments

The time course for association of specific [^{3}H]E2 binding (SB) to cytosolic estrogen receptors revealed as difference from TB and NB (Fig. 1) demonstrated a strong saturating increase during 18 h where maximum binding occurred. Maximum SB remained stable up to 48 h but decreased significantly between 48 and 72 h and therefore all further incubations were carried out with 24 h incubation. No differences could be observed between [^{3}H]E2 binding of male and female liver cytosol.

3.2. Saturation experiments

Specific [^{3}H]E2 binding to liver estrogen receptors was saturable, and NB arose in a nearly linear way (Fig. 2). The corresponding Scatchard-plot indicated that [^{3}H]E2 binding was due to a homogenous receptor population. All saturation curves were very similar, as shown by the relatively small S.E.M. (Fig. 3). Scatchard-plot analyses revealed similar K_d values in males and females with 22.4 ± 6.0 nM and 15.0 ± 2.8 nM (mean $\pm$ S.E.M.; $n = 5$), respectively. However B_{max} showed a bigger number of receptors in females (136 ± 46 fmol [^{3}H]E2/mg protein) com-

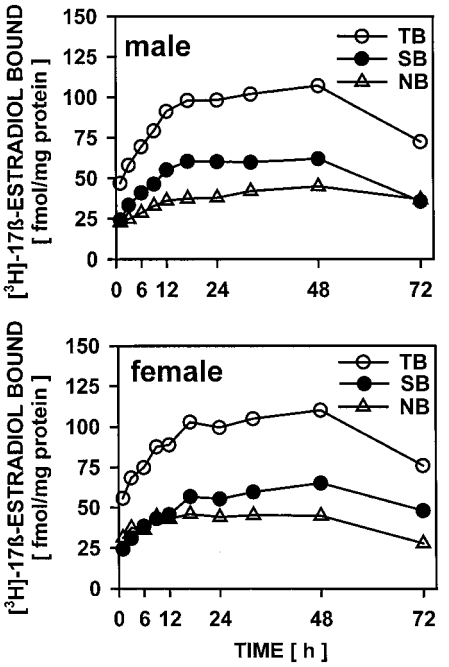

Fig. 1. Time courses of specific [^{3}H]17β-estradiol (E2) binding (SB) revealed as difference of total (TB) and non-specific binding (NB) to estrogen receptors in liver cytosols from male (upper panel) and female (lower panel) *Xenopus laevis*. The association curves of TB were revealed incubating with 13.6 nM [^{3}H]E2 while NB was determined using the same concentration of labeled ligand in the presence of 10^{-5} M unlabeled E2. Points represent mean values of three experiments.

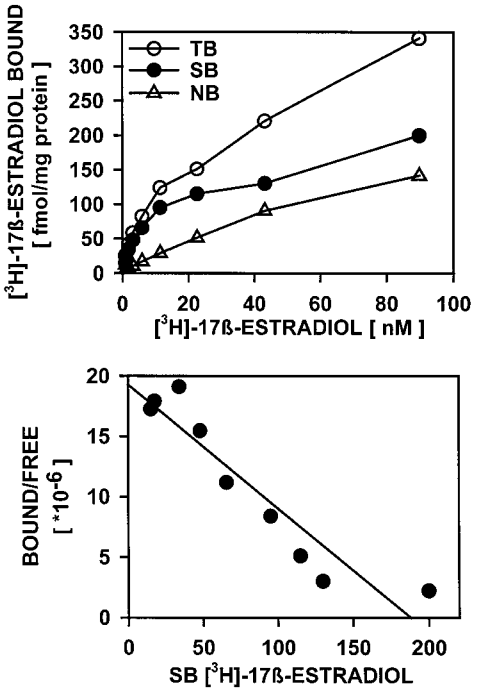

Fig. 2. Saturation curves of TB, SB, and NB for [^{3}H]E2 in liver cytosol of one individual female (upper panel). The lower panel demonstrates the corresponding Scatchard-plot of SB. The slope of the linear regression equals $-1/K_d$ and results in a K_d value of 22.0 nM. B_{max} is obtained by intersection of linear regression and x-axis revealing 183 fmol [^{3}H]E2/mg protein. The linearity of the regression shown by the correlation coefficient (r) of 0.907 indicates the existence of a homogenous population of estrogen receptors.

pared to males (89 ± 46 fmol [^{3}H]E2/mg protein) but this was not significantly different ($P < 0.05$).

3.3. Competitive displacement experiments

Complete displacement of specific [^{3}H]E2 binding by endogenous steroids was only accomplished by unlabeled E2 itself and estrone while DHA had a less pronounced displacing effect (Fig. 4). Other steroids such as Aldo, B, T, and P4 could decrease SB only about 50% at the highest concentration used (10^{-3} M). Results for competitive displacement experiments obtained from male and female liver cytosol did not show any difference concerning specificity of [^{3}H]E2 binding and therefore final statistics of IC_{50} values of endogenous ligands (Table 1) could be calculated as the mean of eight individual experiments. The affinities of endogenous steroids to the estrogen receptor had the following ranking: E2 > estrone > DHA > Aldo ≥ T ≥ B ≥ P4.

Displacement curves of exogenous ligands (Fig. 5) demonstrated that all ligands used were able to compete completely with [^{3}H]E2 binding to estrogen receptors at the highest concentration used (10^{-3} M) except DDT which displaced only about 40% binding under these conditions. Again no differences of specificities could be observed between cytosolic fractions derived from males and females and thus statistical comparisons were performed with a total number of six individuals. The affinity ranking for exogenous ligands demonstrated by IC_{50} values (Table 2) was as follows: E2 > TCB > DEP > BA ≥ NP ≥ HA ≥ OP > DDT.

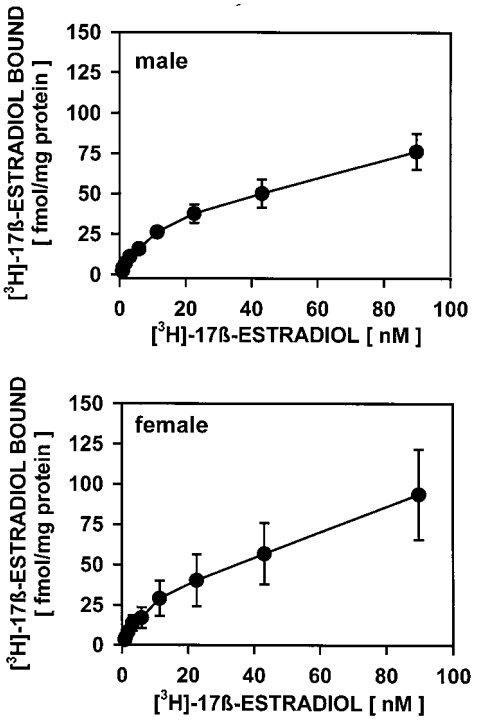

Fig. 3. Mean values ± S.E.M. ($n = 5$) of SB for [^{3}H]E2 from saturation experiments in male (upper panel) and female (lower panel) liver cytosols.

Three out of five sewage effluents demonstrated a clear and dose-dependent displacement of more than 50% of specific [^{3}H]E2 binding using original concentrations (Fig. 6).

4. Discussion

The kinetic experiments for association of [^{3}H]E2 binding to estrogen receptor of *Xenopus laevis* liver cytosol revealed clearly an SB having a nearly identical pattern of time course for males as for females. Maximum binding was reached after 18 h and remained stable up to 48 h indicating that incubations using these assay conditions should be terminated during this period.

Scatchard-plot analyses of saturation experiments resulted always in a homogenous receptor population without statistical differences of binding parameters between both sexes. The K_d-values of males and females were 22.4 ± 6.0 nM and 15.0 ± 2.8 nM (mean ± S.E.M.; $n = 5$), respectively, indicating that *Xenopus laevis* estrogen receptors do not have sex-specific differences concerning their binding affinity. However, the mean B_{max}-values of females (136 ± 46 fmol [^{3}H]E2/mg protein) are higher than those of males (89 ± 46 fmol [^{3}H]E2/mg protein) without having statistical significance only suggesting that females possess more free cytosolic estrogen receptors compared to males. Further experiments using more animals for saturation experiments are needed to reveal a statistical difference between both sexes concerning B_{max}. The finding that male *Xenopus laevis* had only a slightly lower number of estrogen receptors compared to fe-

Table 1
Competitve displacement experiments of [^{3}H]17β-estradiol ([^{3}H]E2) binding by endogenous ligands analyzed using logit–log transformation

Estradiol	36 ± 7
Estrone	59 ± 9
Dehydroepiandrosterone	1726 ± 513
Aldosterone	54 795 ± 28 993
Testosterone	116 958 ± 53 382
Corticosterone	176 720 ± 98 452
Progesterone	193 635 ± 104 671

IC_{50} values [10^{-9} M] (mean ± S.E.M.; $n = 8$). Incubations were carried out using 14.2 nM [^{3}H]E2.

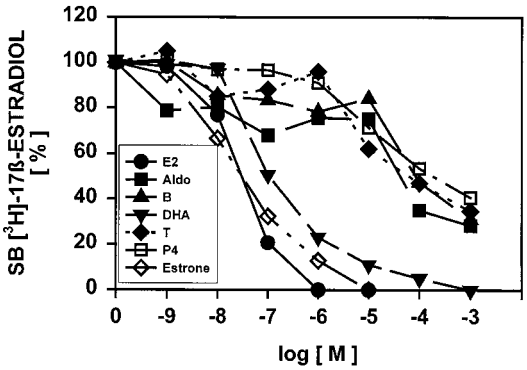

Fig. 4. Mean curves of competitive displacement experiments using various endogenous steroids. Samples from eight individuals were incubated with 14.2 nM [^{3}H]estradiol in the absence or presence of different amounts of unlabeled analogues such as estradiol (E2), aldosterone (Aldo), corticosterone (B), dehydroepiandrosterone (DHA), testosterone (T), progesterone (P4), and estrone. Binding is expressed as percentage of SB. Corresponding IC_{50} values obtained by logit–log transformations are shown in Table 1.

Table 2
Competitve displacement experiments of [^{3}H]17β-estradiol ([^{3}H]E2) binding by environmental chemicals analyzed using logit–log transformation

Estradiol	42 ± 9
Tetrachlorbiphenyl	1708 ± 613
Diethylphthalate	12 483 ± 5527
Bisphenol A	30 198 ± 12 899
Nonylphenol	33 666 ± 15 198
Butylhydroxyanisole	34 961 ± 13 765
Octylphenol	78 320 ± 38 961
Dichlordiphenyltrichlorethan (4,4′-DDT)	1 026 187 ± 798 265

IC_{50} values [10^{-9} M] (mean ± S.E.M.; $n = 6$). Incubations were carried out using 18.9 nM [^{3}H]E2.

males was rather unexpected because the B_{max} of estrogen receptors in females is thought to be much higher than that in males (Tata, 1987) but our preparation allows only estimation of free cytosolic estrogen receptors and no determination of estrogen receptors bound to estrogen or present in the nucleus. Thus the total number of free, estrogen bound, and nuclear estrogen receptors in female liver could be much higher compared to males explaining the relatively small difference of free cytosolic estrogen receptors between both sexes in our experiments. In contrast to our findings Campbell et al. (1994) using a different method of liver cytosol preparation revealed clear significant differences for K_d and B_{max} values of estrogen receptors between both sexes of rainbow trout. Females had higher K_ds of [^{3}H]E2 (9.1 to 12.7 nM) compared to males (2.3 to 4.2 nM) and also B_{max} numbers were much higher (65.5 to 137.3 fmol [^{3}H]E2/mg protein) than those of males (17.3 to 37.2 fmol [^{3}H]E2/mg protein) during several periods over the year. The similarities concerning association of [^{3}H]E2 binding and values of K_d and B_{max} for male as for female liver estrogen receptors in *Xenopus laevis* suggested that both sexes could be used equally to assay estrogen receptor binding of several related and unrelated ligands in competitive displacement experiments.

The specificity of estrogen receptors of *Xenopus laevis* was determined by addition of various endogenous steroids in competitive displacement experiments using four male and four female

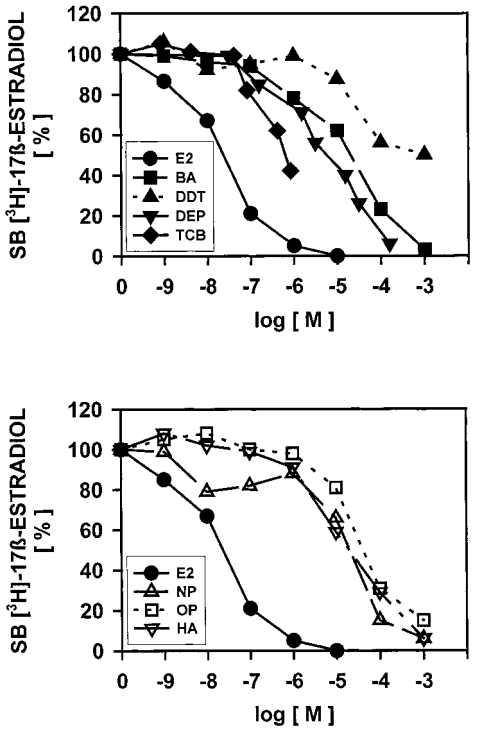

Fig. 5. Competitive displacement of specific [^{3}H]estradiol binding caused by various environmental chemicals in comparison with estradiol (E2). Mean curves ($n = 6$) on the upper panel demonstrate displacing activities of bisphenol A (BA), dichlor-diphenyl-trichlor-ethan (DDT), diethylphthalate (DEP), and tetrachlorbiphenyl (TCB) while the lower panel shows displacements of E2, nonylphenol (NP), octylphenol (OP), and butylhydroxyanisole (HA).

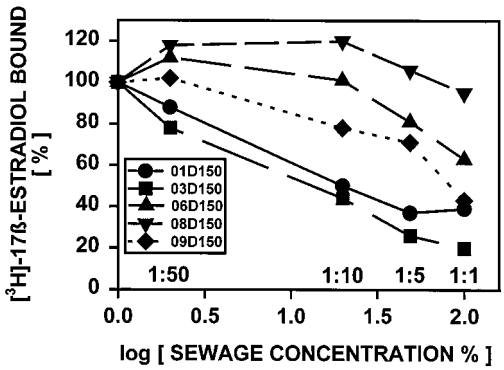

Fig. 6. Individual displacement curves for specific [^{3}H]estradiol ([^{3}H]E2) binding by five randomized samples of sewage effluents from several sources. Dose-dependent competition of [^{3}H]E2 and sewage ingredients for binding to estrogen receptors was achieved by using various dilutions of sterile-filtered samples as indicated in the figure.

individuals. No significant differences of ligand affinities to the estrogen receptor were observed between male and female liver cytosols and therefore results were presented as mean out of eight experiments. Specific [^{3}H]E2 binding was displaced by endogenous steroids with the following affinity ranking: E2 > estrone > DHA > Aldo ≥ T ≥ B ≥ P4. The various affinities of steroids used as shown also by the IC_{50}-values (Table 1) demonstrated that *Xenopus* estrogen receptor is highly specific to the estrogens, E2 and estrone, while DHA has only a moderate binding and all other steroids including the androgen T possess only very weak displacing activities. Thus liver cytosol from both sexes or from several individuals is suggested to be useful for studies determining binding specificity of related and unrelated ligands to the estrogen receptor.

The finding that both sexes could be used in an equal manner to investigate properties of estrogen receptor and its potential ligands was further confirmed by competitive binding studies using exogenous estrogenic compounds for displacement experiments with individual liver cytosol fractions from three males and three females. Again no differences of binding affinities could be observed between both sexes and the ligands used showed an affinity ranking as follows: E2 > TCB > DEP > BA ≥ NP ≥ HA ≥ OP > DDT. These environmental chemicals which are known to be estrogenic in other models, were able to displace specific [^{3}H]E2 binding also from estrogen receptor of *Xenopus laevis*. Compared with the binding of E2 all unrelated ligands used here showed lower binding affinities but all of them except DDT had higher displacing capacities than unrelated endogenous steroids. The present results demonstrate for the first time that environmental chemicals bind also to amphibian estrogen receptors, which is the prerequisite for estrogenic or antiestrogenic biological activity. In rainbow trout a comparable method was used to determine displacement of [^{3}H]E2 binding to estrogen receptor by several environmental chemicals (Jobling et al., 1995). The displacing effects of HA and DEP were much less pronounced in rainbow trout liver cytosol preparations compared to our results obtained from *Xenopus laevis* suggesting a higher sensitivity of the amphibian estrogen receptor to these chemicals. However, the methods applied in rainbow trout and *Xenopus* were different and thus the higher sensitivity of *Xenopus laevis* for estrogen receptor displacement by environmental chemicals could be due just to methodological differences and not to species specific varieties.

The determinations of potential estrogen receptor binding activity in sewage effluents were successful in three out of five randomized samples from various regions. Dose-dependencies confirmed that original composition of sewage effluents were able to cause more than 50% displacement of [^{3}H]E2 binding. In sewage effluents a complex mixture of several chemicals and organic compounds may be present and it seems to be too complicated to perform exact chemical analyses for a complete determination of all ingredients. Therefore the advantage of a bioassay like the radioreceptorassay for estrogen binding is obvious for screening potential estrogenic activities of pure substances as well as of complex mixtures of different compounds. However, detection of binding to estrogen receptor alone does not indicate clearly an estrogenic action of compounds because a bound ligand could also cause antiestrogenic effects by blocking the transactivation via DNA binding. Thus the radioreceptorassay for [^{3}H]E2 binding is a useful method to indicate a potential action of environmental chemicals on estrogen receptors but further experiments are necessary to demonstrate the biological activity in order to detect whether a pure substance or a mixture of compounds are estrogenic or antiestrogenic.

5. Conclusion

In summary the method of radioreceptorassay for [^{3}H]E2 binding in liver cytosol of *Xenopus laevis* was established in order to get a useful tool for detection of potential binding of endocrine disruptors to estrogen receptor in amphibians. The results indicate clearly that liver cytosol of both sexes can be used in an equal way for determinations of estrogen receptor binding because no sex-specific differences could be observed concerning the properties of the estro-

gen receptor. The successful performance of competitive displacement experiments using environmental chemicals as well as sewage effluents suggests that this method can be used for screening of potential effects on estrogen receptors. The question whether pure compounds or complex mixtures of environmental chemicals are estrogenic or antiestrogenic remains to be answered by additional experiments demonstrating induction or inhibition of estrogenic effects in vitro and in vivo.

Acknowledgements

This work was supported by grants (PAÖ 9605.02 and 9702.02) for the project BW-PLUS (Baden-Württemberg-Programm Lebensgrundlage Umwelt und ihre Sicherung) from the Ministry of Environmental Affairs and Traffic Baden-Württemberg. We thank especially Dr Werner Scholz (LFU, Karlsruhe) for many interesting and helpful discussions concerning this topic.

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