Biochemical origins of the non-monotonic receptor-mediated dose–response

M C Kohn and **R L Melnick**

Environmental Toxicology Program, National Institute of Environmental Health Sciences, PO Box 12233, MD A3–06, Research Triangle Park, North Carolina 27709, USA

(Requests for offprints should be addressed to M C Kohn; Email: kohn@niehs.nih.gov)

Abstract

A mathematical model was created to examine how xenobiotic ligands that bind to nuclear receptor proteins may affect transcriptional activation of hormone-regulated genes. The model included binding of the natural ligand (e.g. hormone) and xenobiotic ligands to the receptor, binding of the liganded receptor to receptor-specific DNA response sequences, binding of co-activator or co-repressor proteins (Rp) to the resulting complex, and the consequent transcriptional rate relative to that in the absence of the xenobiotic agent. The model predicted that the xenobiotic could act as a pure agonist, a pure antagonist, or a mixed agonist whose dose–response curve exhibits a local maximum. The response to the agent depends on the affinity of the liganded receptor–DNA complex for binding additional transcription factors (e.g. co-activator proteins). An inverted U-shaped dose–response occurred when basal levels of the natural ligand did not saturate receptor binding sites and the affinity for co-activator is weaker when the xenobiotic ligand is bound to the receptor than when the endogenous ligand is bound. The dose–response curve shape was not dependent on the affinity of the receptor for the xenobiotic agent; alteration of this value merely shifted the curve along the concentration axis. The amount of receptor, the density of DNA response sequences, and the affinity of the DNA-bound receptor for Rp determine the amplitude of the computed response with little overall change in curve shape. This model indicates that a non-monotonic dose–response is a plausible outcome for xenobiotic agents that activate nuclear receptors in the same manner as natural ligands.

Journal of Molecular Endocrinology (2002) **29,** 113–123

Introduction

Nuclear receptors are protein transcription factors that become functionally active on binding of a ligand. When a liganded receptor binds to a particular DNA sequence (response element) near the promoter of a gene, it can modulate the rate of transcription of that gene. Current models of estrogen receptor-regulated transcription suggest that enhanced gene expression results from the recruitment by DNA-bound nuclear receptors of accessory proteins. These include co-activators or co-repressors that interact with the transcription initiation complex (Chen *et al.* 2000) or possess enzymatic activities (e.g. histone acetyltransferase, methyltransferase, ubiquitin ligase) that alter chromatin structure at the promoters of their target genes (Chen *et al.* 2000, McKenna & O'Malley 2000). Dose–response profiles for receptor-mediated processes such as carcinogenesis can exhibit a variety of curve shapes (Hoel *et al.* 1983, Hoel & Portier 1994), including rectangular hyperbolic, sigmoid, and U-shaped (either concave upward or convex upward). The molecular basis for nonlinear dose–response relationships has not been elucidated.

A U-shaped dose–response curve is non-monotonic, i.e. it exhibits a change in the sign of its first derivative. This property can reflect either stimulatory or inhibitory dose-dependent effects on the biological response, according to the mechanistic role of the independent variable. Because current risk assessment procedures are based on the assumption that low-dose responses are linear or threshold appearing, the curve shape in the range of doses expected from environmental exposures

0952–5041/02/029–113

to xenobiotic agents can have important consequences for estimates of the risk of adverse health effects consequent to such exposures.

Mathematical models that accurately represent regulation of biological systems can be used to predict the shape of dose–response curves at low doses. A hypothetical model of the receptor-mediated induction of a protein displayed qualitatively different dose–response behavior when numerical values of its parameters were altered (Kohn & Portier 1993). Positive cooperativity in binding of ligand to the receptor resulted in sigmoid dose–response curves, and positive cooperativity in binding of the liganded receptor to an activating response element-produced U-shaped (concave upward) curves.

Modeling methods

In the present work, a simplified version of that early model (it lacks metabolism of the inducer, protein synthesis, and protein degradation) was constructed for xenobiotic ligands that bind to a nuclear receptor and activate transcription of genes normally regulated by the endogenous ligand. Features of this model are depicted in Fig. 1. Binding of the natural ligand (e.g. 17β-estradiol, E_2) to its receptor ('R' in Fig. 1) induces a conformational change that results in increased binding affinity of the receptor for co-activator proteins ('A'). The transcriptional rate of a reporter gene (luciferase under the control of an estrogen response element) transfected into CV-1 cells was increased by about one order of magnitude when the cells were also transfected with the gene for the co-activator GRIP1 (Chen *et al.* 2000). Therefore, the transcription rate of the target gene in the present model was considered to be proportional to the concentration of DNA-bound liganded receptor that is also bound to co-activator protein.

Co-repressor proteins (Rp) may also bind to the liganded receptor but with different affinities from those of co-activator proteins. This simplified representation of the role of co-activators in mediating nuclear receptor function is intended to reflect the critical binding by the initial transcriptional factor that acts as a scaffold for the recruitment and assembly of the additional co-regulators that mediate expression of the target gene (Chen *et al.* 2000). In this model, if binding of the co-repressor outcompetes binding of co-activator, the resulting complex is not transcriptionally active.

The xenobiotic ligand ('X' in Fig. 1) also binds to the receptor but with lower affinity than the natural ligand. The conformational change induced by the xenobiotic ligand yields a structure with a lower binding affinity for co-activator protein than that of the natural ligand and, possibly, a higher affinity for Rp. Even in the presence of low concentrations of X (Fig. 1A), gene transcription is enhanced because the additional receptors bound with X and with co-activator proteins are transcriptionally active. At high concentrations of X (Fig. 1B), the number of receptors bound with the natural ligand is decreased due to the increased occupation of receptors by X. As R•X binds co-activator more weakly than does $R•E_2$, there may be less transcriptionally competent complex than in the absence of X. This model was used to identify possible mechanistic requirements for the observation of inverted U-shaped (convex upward) dose–response curves.

The cellular concentrations of accessory proteins available to estrogen-responsive genes are unknown, so their actual binding affinities cannot be calculated. However, because the ratio of the binding constant to the accessory protein concentration appears in the differential equations that give the concentrations of the bound proteins needed to calculate the relative transcriptional rate (see below), the normalized non-dimensional value (e.g. $K_{D•R•X•A}=K^*/A$) can be used instead with no loss of generality. EC_{50} values for estrogen receptor-mediated gene expression have been determined for several xenoestrogens, permitting calculation of normalized binding constants for those agents. Some calculated values are E_2, 0·45 (Gaido *et al.* 1997); diethylstilbestrol (DES), 1·77 (Gaido *et al.* 1997); genistein 2·28 (Nikov *et al.* 2000); coumestrol, 2·08 (Nikov *et al.* 2000); nonylphenol, 1·64 (Laws *et al.* 2000); octylphenol, 2·20 (Sheeler *et al.* 2000); bisphenol A, 2117 (Gaido *et al.* 1997); methoxychlor, 18·5 (Gaido *et al.* 1997). No such data are available for Rp, so the corresponding normalized binding constant was a free parameter in this model.

Model structure

The present model is based on Fig. 2. A receptor, 'R', was assumed to bind an endogenous ligand, E_2

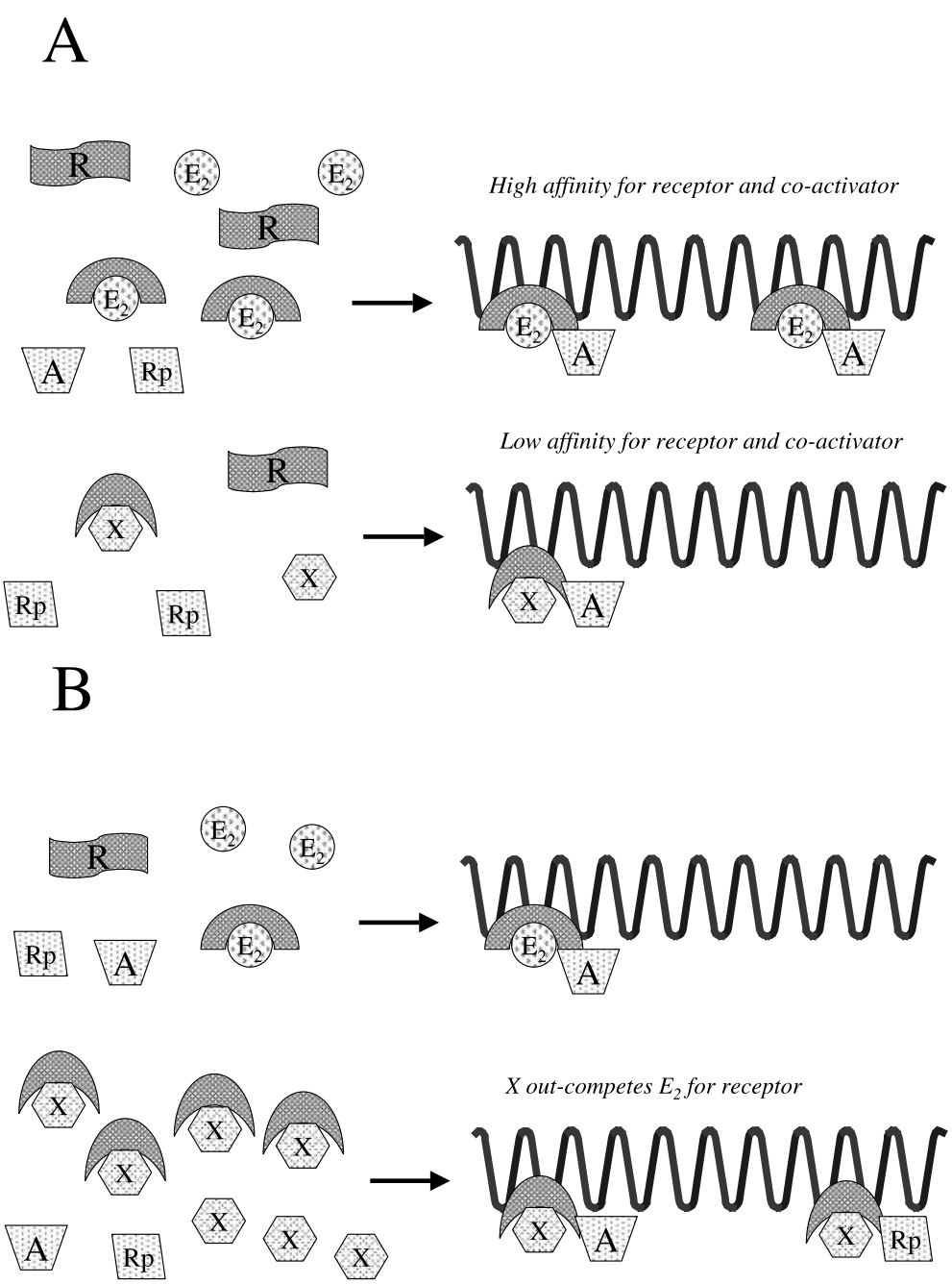

Figure 1 Schematic representation of the mechanism of expression of estrogen-dependent genes. Panel A, low concentration of xenobiotic ligand; panel B, high concentration of xenobiotic ligand. Symbols are R=receptor, E_2=17β-estradiol, A=co-activator, Rp=co-repressor, X=xenobiotic ligand. Note the conformational changes induced in the receptor consequent to ligand binding.

in this model, with a dissociation constant of $K_{R\bullet E2}$ (a). The receptor also can bind a xenobiotic ligand, 'X', with a dissociation constant of $K_{R\bullet X}$ (b). The liganded receptors, R●E_2 and R●X, were assumed to bind to a DNA response element ('D') associated with a target gene with respective dissociation

			Reference
(a) $R + E_2$	$\underset{k2}{\overset{k1}{\rightleftharpoons}}$	$R{\bullet}E_2$	$K_{R\bullet E2} = 0.5$ nM	Hoel & Portier 1994
(b) $R + X$	$\underset{k4}{\overset{k3}{\rightleftharpoons}}$	$R{\bullet}X$	$K_{R\bullet X} = 1000$ nM	Hoel & Portier 1994, Laws *et al* 2000
(c) $R{\bullet}E_2 + D$	$\underset{k6}{\overset{k5}{\rightleftharpoons}}$	$D{\bullet}R{\bullet}E_2$	$K_{RE2\bullet D} = 1$ nM	Lyttle *et al* 1992
(d) $R{\bullet}X + D$	$\underset{k8}{\overset{k7}{\rightleftharpoons}}$	$D{\bullet}R{\bullet}X$	$K_{RX\bullet D} = 1$ nM	Lyttle *et al* 1992
(e) $D{\bullet}R{\bullet}E_2 + A$	$\underset{k10}{\overset{k9}{\rightleftharpoons}}$	$D{\bullet}R{\bullet}E_2{\bullet}A$	$K_{DRE2\bullet A} = 0.5$ nM	Furlow *et al* 1993, McKenna & O'Malley 2000 Murdoch & Gorski 1991
(f) $D{\bullet}R{\bullet}X + A$	$\underset{k12}{\overset{k11}{\rightleftharpoons}}$	$D{\bullet}R{\bullet}X{\bullet}A$	$K_{DRX\bullet A} = 1$ nM (varied in Fig. 4)	
(g) $D{\bullet}R{\bullet}E_2 + Rp$	$\underset{k14}{\overset{k13}{\rightleftharpoons}}$	$D{\bullet}R{\bullet}E_2{\bullet}Rp$	$K_{DRE2\bullet Rp} = 5$ nM	Assumed
(h) $D{\bullet}R{\bullet}X + Rp$	$\underset{k16}{\overset{k15}{\rightleftharpoons}}$	$D{\bullet}R{\bullet}X{\bullet}Rp$	$K_{DRX\bullet Rp} = 1$ nM (varied in Fig. 6)	

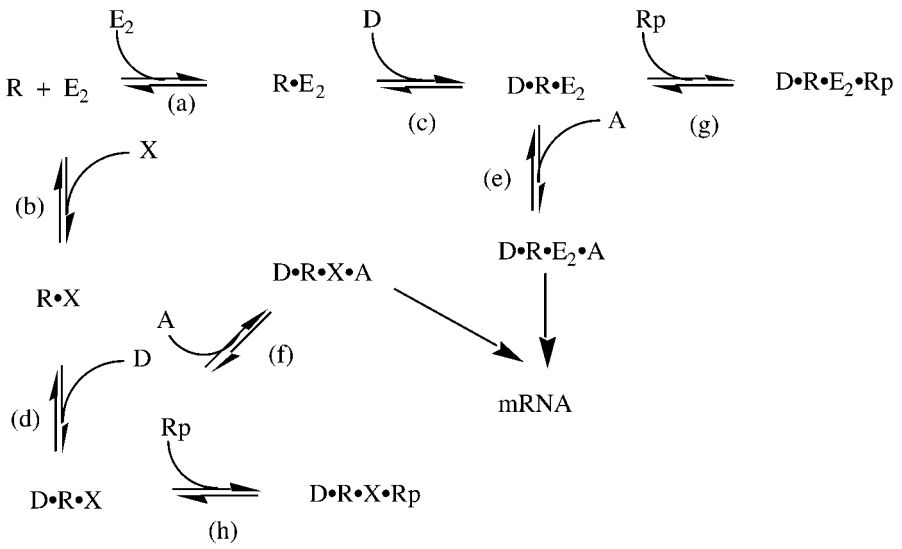

Figure 2 Ligand (E_2=endogenous estradiol, X=xenobiotic) binding to receptor ('R') and liganded receptor binding to DNA enhancer sequence ('D'). Accessory proteins that bind to the liganded receptor–DNA complex are A=co-activator, Rp=co-repressor. The symbol at the end of each line is the dissociation constant for the complex being formed.

constants of $K_{RE2\bullet D}$ (c) and $K_{RX\bullet D}$ (d). The complex formed between the liganded receptor and response element may be stabilized by the binding of accessory protein(s), corresponding to $K_{DRE2\bullet A}$ (e) and $K_{DRX\bullet A}$ (f) for binding of co-activator and $K_{DRE2\bullet Rp}$ (g) and $K_{DRX\bullet Rp}$ (h) for binding of co-repressor respectively. When X is bound to R, the activated receptor can bind to D and induce the same qualitative effects as those induced by E_2. The net rate of transcription, hence the rate of expression of gene product, was assumed to be proportional to the total concentration of co-activator-bound complex ($D\bullet R\bullet E_2\bullet A + D\bullet R\bullet X\bullet A$).

All the equilibria in Fig. 2 occur simultaneously. Assembly of the transcriptional activation complex does not necessarily occur in the specified sequence. For example, a liganded receptor-co-activator complex may form prior to receptor binding to the response element (Margeat *et al.* 2001).

The eight mass action equations describing these simultaneous equilibria involve the calculation of 14 unknown concentrations and four unknown equilibrium constants. This is an unstable, under-determined numerical problem, and no unique solution can be obtained. However, three unknown concentrations could be included in parameters for six equilibrium constants (only their ratios appear in the equations), thus reducing the number of unknowns but still leaving the problem under-determined.

Each equilibrium process

$$\mathrm{L + R} \underset{k_2}{\overset{k_1}{\leftrightarrow}} \mathrm{L \cdot R}$$

$$K_d = \frac{k_2}{k_1}$$

can be written as a set of differential equations given by the law of mass action, e.g.

$$\frac{\mathrm{d[L]}}{\mathrm{d}t} = -k_1[\mathrm{L}][\mathrm{R}] + k_2[\mathrm{L \cdot R}]$$

(L=estrogen receptor ligand)

The differential equations corresponding to Fig. 2 are given in the Appendix.

Starting from arbitrary values for the concentrations, the differential equations can be integrated (*t, time*, is in arbitrary units here) until a steady state is achieved and persists indefinitely. The actual numerical values of the rate constants are irrelevant. Because their ratios equal the equilibrium constants, the exact same solution will be achieved as it would if the eight nonlinear equations could be solved for the 14 unknown concentrations. The numerical values of the rate constants only affect the speed with which this equilibrium is attained. The terminal state will be exactly the one in which all the equilibria are simultaneously satisfied; forward and reverse rates for each reaction are exactly equal, keeping the concentrations of all chemical species constant indefinitely. Equilibrium was established before $t=2$, but the integration was carried to $t=10$ to ensure maintenance of the equilibrium.

The chemical equations were encoded in the SCoP language (Kohn *et al.* 1994) with parameter values comparable to those measured for the estrogen receptor. Estrogen receptor parameters were used in the model because of the greater availability of quantitative data reported for this receptor; however, the qualitative behavior of the model described here is not limited to estrogen receptor-mediated gene activation.

Although estrogen receptor dimerization is necessary for transcription (Wang *et al.* 1995, Margeat *et al.* 2001), the corresponding dissociation constant is small. Half maximal dimerization was observed at 0·12 nM E_2 (Sheeler *et al.* 2000), indicating that most of the liganded receptor would exist as dimers even at minimal levels of E_2 in adult females. In addition, binding of the estrogen receptor to estrogen response elements (EREs) may (Boyer *et al.* 2000) or may not (Murdoch & Gorski 1991) display cooperativity. Furthermore, there is evidence (Boyer *et al.* 2000) that estrogen receptors can bind to EREs as monomers and form dimers *in situ*. Therefore, a separate step for receptor dimerization would have no effect on the behavior of the model and was omitted from this simplified model.

Similarly, each receptor monomer in the dimer may be bound to E_2 or X. It is unknown how most xenoestrogens affect the binding constants for accessory proteins. If the affinity of a mixed dimer for accessory proteins were the average of the affinities of the homodimers, including dimeriz-

ation would have no effect on the numerical output of the model.

The dissociation constants were set to $K_{R\bullet E2}=0{\cdot}5$ nM for E_2 (Laws *et al.* 2000), $K_{R\bullet X}=1000$ nM (comparable to the values (Dodge *et al.* 1996, Laws *et al.* 2000) for several weakly estrogenic chemicals), and $K_{RE2\bullet D}=K_{RX\bullet D}=1$nM (Inano *et al.* 1994). The binding affinity of the estrogen receptor for EREs appears to be independent of the ligand bound to the receptor, and may occur in the absence of ligand (Curtis & Korach 1991, Inano *et al.* 1994). As the intracellular concentrations of co-activators and co-repressors are unknown, these quantities were divided into their respective dissociation equilibrium constants, resulting in a dimensionless quantity. $K_{DRE2\bullet A}$ was fixed at 0·5 (Gaido *et al.* 1997, Suen *et al.* 1998, Thénot *et al.* 1999), and $K_{DRX\bullet A}$ is a parameter in the model. In the absence of data, $K_{DRE2\bullet Rp}$ was fixed at 5, and $K_{DRX\bullet Rp}$ is a parameter in the model, whose effect was studied by computer experiments in which its value was systematically varied.

The initial concentrations of E_2 and R were respectively set to 0·2 nM (blood E_2 concentration in female rats) and 10 nM (Furlow *et al.* 1993). If E_2 equilibrates between serum and tissue, use of the blood hormone level serves as the upper bound of tissue concentrations. The initial concentration of E_2 relative to the concentration of R ensures that unoccupied receptors are present in excess over the endogenous ligand. The value for D is unknown. Therefore, it was set to 1 so that the solution of the equations corresponded to the fraction of D that is transcriptionally active.

Using the law of mass action, the above scheme was converted into differential equations. These equations were integrated with SCoP (Kootsey *et al.* 1986) from *time*=0 to *time*=10 for each of several initial concentrations of X to distribute ligand and receptor among their free and bound forms and to achieve the corresponding steady-state solutions. The steady-state value of

$$v_{\text{relative transcription}} = \frac{[D\bullet R\bullet E_2\bullet A](X)+[D\bullet R\bullet X\bullet A](X)}{[D\bullet R\bullet E_2\bullet A](X=0)}$$

gives the effect (relative to X=0) of X on the expression rate of a gene regulated by E_2.

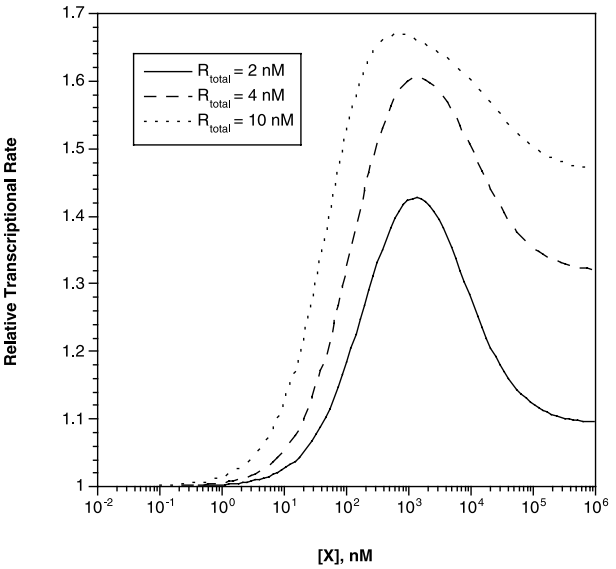

Figure 3 Effect of total amount of estrogen receptor on relative transcriptional rate. Other parameter values are as given in Fig. 2. Although the response is not directly proportional to receptor concentration because of saturation at lower receptor concentrations, the effect is mainly on the amplitude of the response and not on the curve shape.

Implicit in this formulation is the notion that the scaffold of proteins assembled on the response element has the same effectiveness at inducing transcription regardless of the receptor ligand that initiated the process. Each ligand alters the affinities of the bound receptor for accessory proteins to a different extent as shown by Kraichely *et al.* (2000).

Results

Receptor-mediated responses are routinely observed at ligand concentrations far below those that result in occupation of most of the receptor binding sites. Under most conditions response elements are occupied to a small extent, and saturation of the cellular response occurs at receptor titers far above those found naturally (Webb *et al.* 1992). This is the origin of the concept of 'spare receptors,' and is illustrated by the model's predictions in a computer experiment (Fig. 3).

The initial concentration of R was varied (Fig. 3, R=2, 4 or 10 nM). In each of these cases there were unoccupied ligand-binding sites (E_2=0·2 nM)

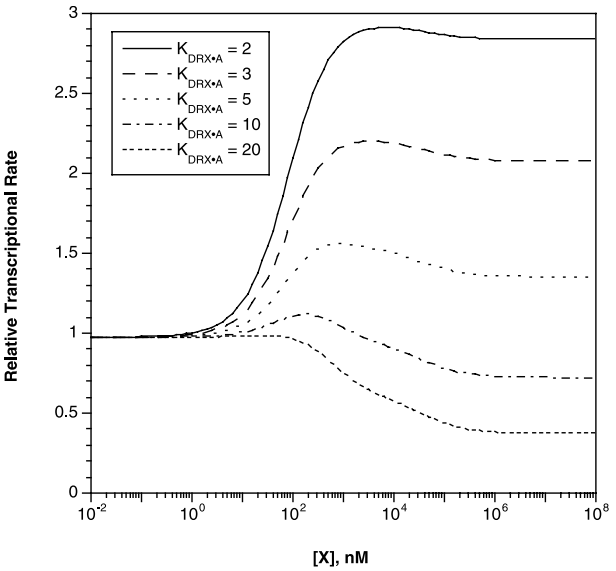

Figure 4 Dose–response profiles for relative transcriptional rate at different affinities ($K_{DRX•A}$) of the DNA-bound liganded receptor (D•R•X) for the co-activator ('A'). The xenobiotic agent can act as a pure agonist, a pure antagonist, or a mixed agonist depending on the affinity. $K_{DRX•Rp}$=5. Other parameter values are given in Fig. 2.

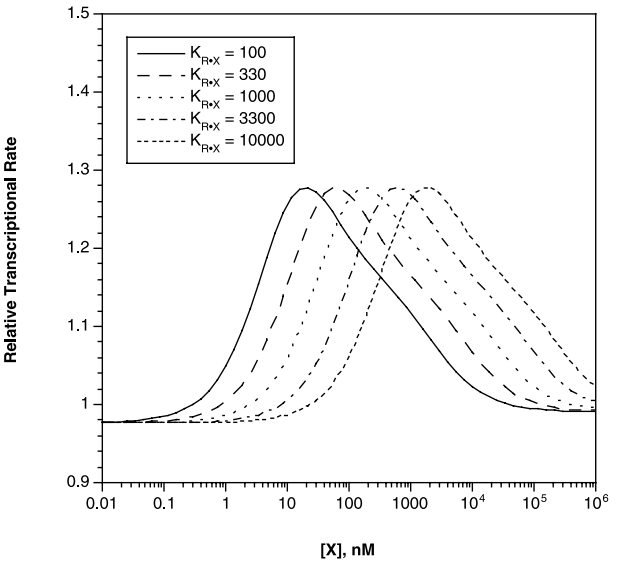

Figure 5 Altering the affinity of the receptor for the xenobiotic ligand ('X') does not affect the shape of the dose–response curve. It merely shifts the maximum of the curve to higher or lower concentrations. $K_{DRX•A}$=10; $K_{DRX•Rp}$=5.

in the absence of X. As a result, all three dose–response curves exhibit an inverted-U shape, and the maximal response was increased with increasing receptor concentration. Elimination of the inverted U-shaped dose–response curve was achieved by increasing the concentration of E_2 to the point that all ligand-binding sites of R were bound with E_2. With no unoccupied receptors, additional transcriptional activation was impossible and X was predicted to be a pure antagonist. As the total receptor concentration changes, the response changes in amplitude but not in curve shape. The response is not in direct proportion to receptor concentration because saturation of response elements occurs at normally available receptor concentrations. As the transcriptional rate is limited by availability of response elements, supplying additional receptors does not greatly increase the rate.

The model developed here produced an inverted U-shaped dose–response curve as the total tissue concentration of X increased from 1 to 10^4 nM (Fig. 4, especially curves with $K_{DRX•A}$=5–10). This behavior requires unoccupied receptors in the presence of endogenous ligand E_2, i.e. free receptors are available at normal levels of endogenous hormone. At low concentrations of X (between 1 and 100 nM), some of the unoccupied receptors become liganded with X, and expression of the responsive gene is predicted to increase. This is true even for xenoestrogens that have a weak affinity for R. In this model the binding affinity of X is 2000 times weaker than that of E_2 for R. At high concentrations of X (above 1000 nM), the xenoestrogen out-competes E_2 for D. Because $K_{DRX•A} >> K_{DRE2•A}$, there is less liganded receptor–DNA response element complexes bound with co-activator, and the net receptor-mediated effect is reduced. If A has a high affinity for the D●R●X complex, X behaves like a pure agonist (Fig. 4, $K_{DRX•A}$=2). If A has a low affinity, such that Rp out-competes A for binding to the receptor–DNA complex, X behaves like a pure antagonist (Fig. 4, $K_{DRX•A}$=20).

In contrast to changes in $K_{DRX•A}$, changing $K_{R•X}$ did not alter the shape of the dose–response curve (Fig. 5). Increasing $K_{R•X}$ (decreasing the affinity of X for R) shifts the curve to the right, whereas decreasing $K_{R•X}$ (increasing the affinity of X for R) shifts the curve to the left. Figure 5 represents responses that may be expected from xenoestrogens that differ only in their affinity for R.

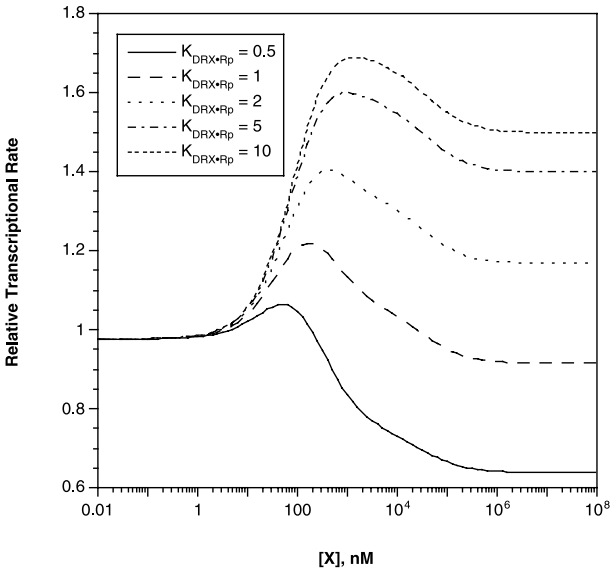

Figure 6 Influence of $K_{DRX\cdot Rp}$ on the dose–response curve shape. $K_{DRX\cdot A}$=10; $K_{R\cdot X}$=1000.

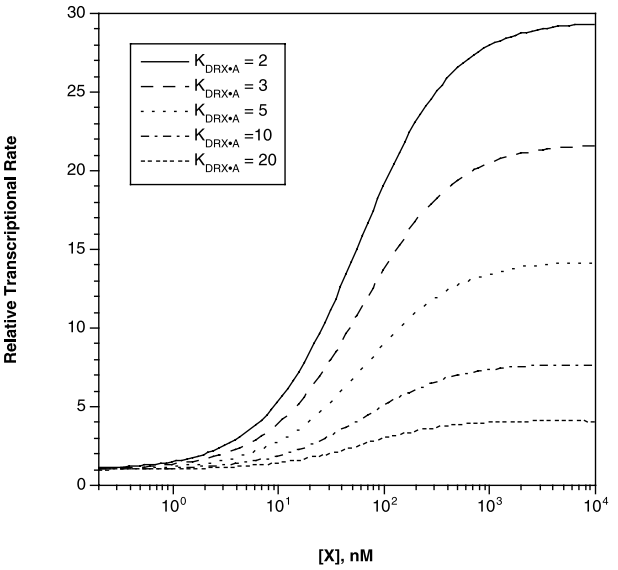

Figure 7 Effect of K_{DRX} on relative transcriptional rate when E_2=0·02 nM (serum concentration in men and juveniles). At these low estradiol levels, there are so many unoccupied receptors that X acts as an agonist even when co-activator binding is weak ($K_{DRX\cdot A}$=20). Other parameter values are given in Fig. 2.

When the concentration of D (initially=1nM) was varied from 0·5 to 10, it was still possible to identify parameter values that predicted X to be either a pure agonist or a pure antagonist or to produce an inverted U-shaped dose–response. The effect was merely to alter the amplitude of the response curve.

At high affinity for co-repressor (Fig. 6, $K_{DRX\bullet Rp}$=0·5) the xenobiotic ligand acts mainly as an antagonist, and at low affinity (Fig. 6, $K_{DRX\bullet Rp}$= 10) it acts mainly as an agonist. However, this qualitative difference is largely due to alteration of the amplitude of the response. By competing with co-activator for binding to the D●R complex, the co-repressor reduces the amount of transcriptionally competent complex present, and thus the rate of transcription decreases.

Discussion

The present model predicts that inverted U-shaped responses to xenobiotic ligands of transcriptionally active receptors require the existence of unoccupied receptors that are available to bind with X. Serum-free (not bound to steroid-binding globulin) E_2 in normally cyclic women rises from 0·16 nM early in the follicular stage to 0·44 nM (maximal mean value reported is 0·8 nM) in mid-cycle (Sehested *et al.* 2000). As the estrogen receptor K_d for E_2 is 0·5 nM, the receptor is unlikely to be saturated even at mid-cycle. For young girls, the existence of unoccupied receptors is even clearer. Circulating E_2 in prepubescent girls has been measured (Ankarberg & Norjavaara 1999) at 0·01–0·02 nM, rising to 0·1–0·8 nM at the end of puberty. As the maximal occupation of the receptor in the absence of cooperative binding of ligands (calculated from the Michaelis–Menten equation) is about 60%, the existence of unoccupied receptors appears to be generally true.

Circulating E_2 is also lower in men and boys. The model's predictions when E_2=0·02 nM are given in Fig. 7. Even when co-activator binding is weak ($K_{DRX\bullet A}$=20), the xenoestrogen acts as a pure agonist. This behavior is due to the existence of a large number of unoccupied receptors in the absence of X that is available for binding the xenoestrogen. At low E_2 (0·02 nM, Fig. 7) the absolute transcriptional rate is low in the absence of X, and the relative rate increase in the presence of X is much larger than at high E_2 (0·2 nM, Fig. 4). Thus, men and prepubescent boys and girls may be more susceptible to the effects of xenoestrogens.

The model predicts that as X increases, the unoccupied receptors become liganded and the

transcriptional signal increases beyond that resulting from the endogenous ligand alone. At high concentrations of X, the xenobiotic agent will out-compete E_2 for R, and most of the receptor will be bound with X. As the affinity of D●R●X for co-activator is lower than that of D●R●E_2, the concentration of D●R●E_2●A will be lower than it would be in the absence of X. Consequently the rate of expression will be lower.

This behavior was not qualitatively altered by changes in receptor concentration (as long as R exceeds E_2), the affinity of the receptor for the xenobiotic ligand ($K_{R\bullet X}$), or the concentration of response elements (as long as these elements are not saturated with receptor in the absence of xenobiotic ligand). When different $K_{R\bullet X}$ values representative of xenobiotic agents that activate R – DES, 0·2 nM; ethynylestradiol, 0·4 nM; nonylphenol, 672 nM; octylphenol, 781 nM; bisphenol A, 1·57 μM; methoxychlor, 65 μM (Laws *et al.* 2000) – were used in the model, the shape of the dose–response curve was not changed.

The primary effect of a change in $K_{R\bullet X}$ is a shift in the positions on the X concentration axis of the maximum of the dose–response curve. Similarly, variation in the affinity for co-repressor affected mainly the amplitude of the response rather than the qualitative effect of the xenobiotic ligand. Only the affinity of the enhancer-bound liganded receptor for co-activator ($K_{DRX\bullet A}$) strongly affected the shape of the dose–response curve. If $K_{D\bullet Rp}$ is decreased (i.e. greater affinity for co-repressor), the xenoestrogen would appear primarily antagonistic.

Such a shift between agonistic and antagonistic behavior has been observed in yeast transfected with genes for estrogen receptor and a β-galactosidase gene under the control of an ERE. Thus, the alternative ligands ICI 164,384, ICI 182,780, and tamoxifen activate transcription at low doses but diminish transcription at high doses (Lyttle *et al.* 1992, Wang *et al.* 1995).

Although this model is extremely simple, the robustness of its predictions illustrates the plausibility of inverted U-shaped dose–response profiles for receptor-mediated processes. As $K_{DRX\bullet A}$ values are unknown for most xenobiotic agents that transcriptionally activate nuclear hormone receptors, this model has identified a critical research need. These results indicate that quantitative measurement of the mechanistic events involved in receptor-mediated responses, the concentrations of the various components of the system (e.g. E_2, X, D, A, Rp), and the dissociation constants given in Fig. 2 are crucial to reliably predict effects from exposures to xenobiotic ligands that transcriptionally activate that receptor.

References

Ankarberg C & Norjavaara E 1999 Diurnal rhythm of testosterone secretion before and throughout puberty in healthy girls: correlation with 17β-estradiol and dehydroepiandrosterone sulfate. *Journal of Clinical Endocrinology and Metabolism* **84** 975–984.

Boyer M, Poujol N, Margeat E & Royer CA 2000 Quantitative characterization of the interaction between purified human estrogen receptor α and DNA using fluorescence anisotropy. *Nucleic Acids Research* **28** 2494–2502.

Chen D, Huang S-M & Stallcup MR 2000 Synergistic, p160 coactivator-dependent enhancement of estrogen receptor function by CARM1 and p300. *Journal of Biological Chemistry* **275** 40810–40816.

Curtis SW & Korach KS 1991 Uterine estrogen receptor–DNA complexes: effects of different ERE sequences, ligands, and receptor forms. *Journal of Molecular Endocrinology* **5** 959–966.

Dodge JA, Glasebrook AL, Mages DE, Phillips DL, Sato M, Short LL & Bryant HU 1996 Environmental estrogens. Effects on cholesterol lowering and bone in the ovariectomized rat. *Journal of Steroid Biochemistry and Molecular Biology* **59** 155–161.

Furlow JD, Murdoch FE & Gorski J 1993 High affinity binding of the estrogen receptor to a DNA response element does not require homodimer formation or estrogen. *Journal of Biological Chemistry* **268** 12519–12525.

Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ & McDonnell DP 1997 Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicology and Applied Pharmacology* **143** 205–212.

Hoel DG & Portier CJ 1994 Nonlinearity of carcinogenesis dose–response functions. *Environmental Health Perspectives* **102** (Suppl) 109–113.

Hoel DG, Kaplan NL & Anderson MW 1983 Implication of nonlinear kinetics on risk estimation in carcinogenesis. *Science* **219** 1032–1037.

Inano K, Curtis SW, Korach KS, Omata S & Horigome T 1994 Heat shock protein 90 strongly stimulates the binding of purified estrogen receptor to its responsive element. *Journal of Biochemistry* **116** 759–766.

Kohn MC & Portier CJ 1993 Effects of the mechanism of receptor-mediated gene expression on the shape of the dose–response curve. *Risk Analysis* **13** 565–572.

Kohn MC, Hines ML, Kootsey JM & Feezor MD 1994 A block organized model builder. *Mathematical and Computer Modelling* **19** 75–97.

Kootsey JM, Kohn MC, Feezor MD, Mitchell GR & Fletcher PR 1986 SCoP: an interactive simulation control program for micro- and minicomputers. *Bulletin of Mathematical Biology* **48** 427–441.

Kraichely DM, Sun J, Katzenellenbogen JA & Katzenellenbogen BS 2000 Conformational changes and coactivator recruitment by novel ligands for estrogen receptor-α and estrogen receptor-β: correlations with biological character and distinct differences among SRC coactivator family members. *Endocrinology* **141** 3534–3545.

Laws SC, Carey SA, Ferrell JM, Bodman KM & Cooper RL 2000 Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. *Toxicological Sciences* **54** 154–167.

Lyttle CR, Damian-Matsumura P, Juui H & Butt TR 1992 Human estrogen receptor regulation in a yeast model system and studies on receptor agonists and antagonists. *Journal of Steroid Biochemistry and Molecular Biology* **42** 677–685.

McKenna NJ & O'Malley BW 2000 From ligand to response: generating diversity in nuclear receptor coregulator function. *Journal of Steroid Biochemistry and Molecular Biology* **74** 351–356.

Margeat E, Poujol N, Boulahtouf A, Chen Y, Müller JD, Gratton E, Cavaillès V & Royer CA 2001 The human estrogen receptor α dimer binds a single SRC-1 coactivator molecule with an affinity dictated by agonist structure. *Journal of Molecular Biology* **306** 433–442.

Murdoch FE & Gorski J 1991 The role of ligand in estrogen receptor regulation of gene expression. *Molecular and Cellular Endocrinology* **78** C103–C108.

Nikov GN, Hopkins NE, Boue S & Alworth WL 2000 Interactions of dietary estrogens with human estrogen receptors and the effect on estrogen receptor–estrogen response element complex formation. *Environmental Health Perspectives* **108** 867–872.

Sehested A, Juul A, Andersson AM, Petersen JH, Jensen TK, Müller J & Skakkerbaek NE 2000 Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. *Journal of Clinical Endocrinology and Metabolism* **85** 1634–1640.

Sheeler CQ, Dudley MW & Khan SA 2000 Environmental estrogens induce transcriptionally active estrogen receptor dimers in yeast: activity potentiated by the coactivator RIP140. *Environmental Health Perspectives* **108** 97–103.

Suen CS, Berodin TJ, Mastroeni R, Cheska BJ, Lyttle CR & Frail DE 1998 A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. *Journal of Biological Chemistry* **273** 27645–27653.

Thénot S, Bonnet S, Boulahtouf A, Margeat E, Royer CA, Borgna J-L & Cavaillès V 1999 Effect of ligand and DNA binding on the interaction between human transcription intermediary factor 1α and estrogen receptors. *Journal of Molecular Endocrinology* **13** 2137–2150.

Wang H, Peters FA, Zeng X, Tang M, Ip W & Khan SA 1995 Yeast two-hybrid system demonstrates that estrogen receptor dimerization is ligand-dependent. *Journal of Biological Chemistry* **270** 23322–23329.

Webb P, Lopez GN, Greene GL, Baxter JD & Kushner PJ 1992 The limits of the cellular capacity to mediate an estrogen receptor. *Journal of Molecular Endocrinology* **6** 157–167.

Received in final form 26 February 2002
Accepted 27 March 2002

Appendix

$$\frac{d[R]}{dt} = -k1[R][E_2] + k2[R \bullet E_2] - k1[R][E_2] + k2[R \bullet E_2]$$

$$\frac{d[R \bullet E_2]}{dt} = k1[R][E_2] - k2[R \bullet E_2]$$

$$\frac{d[E_2]}{dt} = -k1[R][E_2] + k2[R \bullet E_2]$$

$$\frac{d[X]}{dt} = -k3[R][X] + k4[R \bullet X]$$

$$\frac{d[D]}{dt} = -k5[R \bullet E_2][D] + k6[D \bullet R \bullet E_2] - k7[R \bullet X][D] + k8[D \bullet R \bullet X]$$

$$\frac{d[D \bullet R \bullet E_2]}{dt} = k5[R \bullet E_2][D] - k6[D \bullet R \bullet E_2]$$

$$\frac{d[D \bullet R \bullet X]}{dt} = k7[R \bullet X][D] - k8[D \bullet R \bullet X]$$

$$\frac{d[A]}{dt} = -k9[D \bullet R \bullet E_2][A] + k10[D \bullet R \bullet E_2 \bullet A] - k11[D \bullet R \bullet X][A]$$

$$\frac{d[D \bullet R \bullet E_2 \bullet A]}{dt} = k9[D \bullet R \bullet E_2][A] - k10[D \bullet R \bullet E_2 \bullet A]$$

$$\frac{d[D \bullet R \bullet X \bullet A]}{dt} = k11[D \bullet R \bullet X][A] - k12[D \bullet R \bullet X_2 \bullet A]$$

$$\frac{d[D \bullet R \bullet E_2 \bullet Rp]}{dt} = k9[D \bullet R \bullet E_2][Rp] - k10[D \bullet R \bullet E_2 \bullet Rp]$$

$$\frac{d[D \bullet R \bullet X \bullet Rp]}{dt} = k11[D \bullet R \bullet X][Rp] - k12[D \bullet R \bullet X_2 \bullet Rp]$$

$$\frac{d[Rp]}{dt} = -k9[D \bullet R \bullet E_2][Rp] + k10[D \bullet R \bullet E_2 \bullet Rp]$$