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Classical Nuclear Hormone Receptor Activity as a Mediator of Complex Concentration Response Relationships for Endocrine Active Compounds

Clifford J. Cookman and **Scott M. Belcher**
Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH, 45267-0575

Abstract

Nonmonotonic concentration response relationships are frequently observed for endocrine active ligands that act via nuclear receptors. The curve of best fit for nonmonotonic concentration response relationships are often inverted U-shaped with effects at intermediate concentrations that are different from effects at higher or lower concentrations. Cytotoxicity is a major mode of action responsible for inverted U-shaped concentration response relationships. However, evidence suggests that ligand selectivity, activation of multiple molecular targets, concerted regulation of multiple opposing endpoints, and multiple ligand binding sites within nuclear receptors also contribute to nonmonotonic concentration response relationships of endocrine active ligands. This review reports the current understanding of mechanisms involved in classical nuclear receptor mediated nonmonotonic concentration response relationships with a focus on studies published between 2012 and 2014.

Keywords

androgen receptor; bisphenol A; concentration response; endocrine disruptor; estrogen receptor; nonmonotonic dose response; phytoestrogen; PPARγ; thyroid hormone

Introduction

Complex concentration response (C/R) relationships are observed for a variety of compounds including micronutrients, endocrine disrupting chemicals (EDCs) and endogenous hormones. Any C/R relationship whose curve of best fit has a slope that changes sign (direction) along a defined dose range would classify as a nonmonotonic C/R curve. Over the concentration range examined in many studies, these nonmonotonic C/R

Corresponding Author: Scott M. Belcher, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, 231 Albert Sabin Way; PO Box 670575, Cincinnati, OH, 45267-0575, Telephone: 513-558-1721, scott.belcher@uc.edu.

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curves are often observed as an inverted U-shaped curve characterized by an intermediate dose having an effect that is not observed at higher or lower doses. However, nonmonotonic C/R curves can also possess multiple inflection points indicating additional complexity [1,2].

Nuclear receptors (NRs) “classically” function as ligand-activated transcription factors that act in the nucleus following binding of an endogenous hormone or environmental ligand. The ligand bound NR interacts with specific DNA sequences termed hormone responsive elements (HRE) and, in cooperation with co-regulator proteins, regulate assembly of multi-protein transcriptional complexes to modulate transcription of responsive genes (Fig. 1). These co-regulators include co-activators and co-repressors that facilitate multi-protein complex assembly with NRs to enhance (Fig. 1A) or repress (Fig. 1B) target gene expression [3]. Nuclear receptors can also modulate transcriptional activity through ligand independent mechanisms involving receptor specific phosphorylation (Fig. 1C). For estrogen receptor α (ERα), phosphorylation of the AF1 domain (serine 118) results in constitutive ERα transcriptional activity independent of estrogen binding [4]. Binding of ligand at a constitutively active NR may act as an inverse agonist resulting in decreased activities (Fig. 1D). The thyroid hormone receptor (TR), which is constitutively bound to its TRE and represses transcription until binding of ligand, can also be considered as a negative form of constitutive activity [5].

In addition to acting via these classical nuclear hormone receptor mechanisms, several of the nuclear receptors, including the estrogen, androgen and progesterone receptors, act in the cytoplasm or associated with the plasma membrane to participate in rapid intracellular signaling mechanisms independent of the classical nuclear receptor transactivational pathway [6]. Nuclear estrogen receptors can also modulate of the nuclear receptor activity of NFκB and interact with Sp1 and AP1 transcription factors to affect the expression of responsive genes [7–9].

Nonmonotonic C/R relationships: mechanisms and modes of actions

Many studies both *in vitro* and *in vivo* have reported nonmonotonic C/R relationships for endocrine active compounds such as endogenous hormones and EDCs. A variety of potential mechanisms of action have been hypothesized as contributing to the complex relationships observed for endocrine active compounds and include high-dose induction of cytotoxicity, receptor ligand selectivity, differential expression of receptors and co-regulators, ligand-induced receptor down-regulation, competition between multiple receptors, and endocrine negative feedback loops [2].

Cytotoxic effects at high concentrations of NR ligands are the most common mode of action responsible for nonmonotonic C/R relationships in experimental studies, and are often the result of non-specific (off-target) effects. However, when assessing the biological effects of EDCs, ligand selectivity for different NRs is also an important consideration because several EDCs can bind multiple NRs depending on the concentration. For example, bisphenol A (BPA) selectively binds and activates ERβ ($K_i \approx 35$ nM) and ERα ($K_i \approx 195$ nM), but will also bind and inhibit the androgen ($K_i \approx 18$ μM) and thyroid hormone ($K_i \approx 100$ μM)

receptors at higher concentrations [10–13]. It is easy to understand how receptor selectivity can cause nonmonotonic C/R relationships when one considers that the biological effect of the different receptors may oppose each other. The ERs serve as an especially good example of opposition between NR activities. When coexpressed, ERα activation stimulates proliferation in the prostate and uterus, while activation of ERβ opposes the proliferative effects of ERα [2,14].

Cytotoxicity induced nonmonotonic C/R relationships

Cytotoxicity-induced complex C/R relationships are common and result when a compound initially causes a biological effect through specific binding at a NR, but also induces non-specific (NR-independent) cytotoxic effects at higher concentrations which counteract the specific NR-mediated effects. This phenomenon occurs in response to the stilbenoid ER ligand resveratrol, and the isoflavonoid phytoestrogens daidzein and genistein [15,16]. Resveratrol at concentrations from 0.1 to 1 μM has mitogenic actions in the GH3 pituitary tumor cell line, but concentrations above 10 μM results in increased caspase-3 activity and decreased cell numbers [15]. Genistein and daidzein have similar effects on mitogenesis in MCF-7 breast cancer cells, where mitogenic effects were observed in response to 1 and 10 μM, and 200 μM resulted in decreased viable cell numbers [16]. In this same study increasing concentrations of either genistein or daidzein also decreased viable cell numbers in ZR-75-1 or SK-BR-3 breast cancer cells. This effect was associated with increased apoptosis in ZR-75-1 cells [16]. The mitogenic effects at 1–10 μM in MCF-7 cells were interpreted as being mediated by ERα; however, the lack of mitogenic effects in the ERα positive ZR-75-1 cells suggests that factors in addition to ER status influence the mitogenic actions of genistein and daidzein [16]. The apoptotic effects of these phytoestrogens were suggested to result from decreased expression of the epidermal growth factor family tyrosine kinase ERBB2. However, it is notable that high concentrations of these phytoestrogens also decreased viable MCF-7 cell number, even though these breast cancer cells that do not express ERBB2, a finding also suggesting that alternative or more complex mechanisms are involved in these processes [16]. A likely explanation for the antiproliferative/cytotoxic actions of genistein is that along with being an ER ligand, genistein is also an inhibitor of receptor tyrosine kinases [17]. Receptor tyrosine kinase activity blockade at higher concentrations of genistein are expected to decrease mitogenesis and increase apoptosis in these breast cancer cells, effects considered likely to have contributed to the observed nonmonotonic C/R relationship.

Studies investigating the effects of BPA and genistein on prostate cancer cell proliferation and the effects of danazol on endothelial cell permeability, also support the idea that cytotoxicity contributes to C/R complexity [18,19]. Initial studies investigating the effect of BPA on prostate cancer cell proliferation demonstrated that the effect of BPA depended on the mutational status of the androgen receptor (AR). Prostate cancer cells that express the mutant AR-T877A allele are androgen dependent and BPA can increase proliferation through the activation of the mutant AR [20,21]. More recent studies have confirmed that mutational status affects AR ligand binding and activation by demonstrating that genistein also activates AR-T877A but not the wildtype AR. In those studies, genistein could increase LNCaP cell proliferation in the concentration range of 0.5 to 5 μM and inhibited

proliferation at concentrations above 25 μM. In contrast, proliferation of androgen dependent LAPC-4 and androgen independent PC-3 cells was inhibited by increasing concentrations genistein in a monotonic concentration dependent manner. These differential effects were the result of mutant AR activation by genistein at low concentrations, while off-target increases in cytotoxicity are likely responsible for decreased proliferation at higher concentrations [19].

In a similar fashion, the synthetic androgen danazol at concentrations from 100 to 500 nM can decrease human umbilical vein endothelial cell (HUVEC) permeability [18]. Other tested concentrations did not affect HUVEC permeability except the highest tested concentration (50 μM) which increased HUVEC permeability. The decreased permeability of HUVECs in response to 100 nM danazol was due to AR activation since the AR antagonist hydroxyflutamide blocked this effect. The mechanism responsible for the increased HUVEC permeability at the high danazol concentrations was not investigated but appears likely to have resulted from non-specific effects [18]. Because danazol can decrease HUVEC viability at concentrations above 10 μM, the nonmonotonic C/R relationship observed for HUVEC permeability was likely due to the cytotoxic effects of high concentration danazol [22].

While cytotoxicity at high concentrations of NR ligands can be responsible for an observed complex C/R relationship, additional factors can be involved. The involvement of additional contributing factors was evident in our recent examination of peroxisome proliferator-activated receptor γ (PPARγ) activity in response to the flame retardant mixture Firemaster® 550 (FM 550) [23]. FM 550 increased PPARγ activity with maximal stimulation observed at 25 μM; however, agonist effects at PPARγ were lost at higher concentrations. Nonmonotonic C/R relationships were also observed in response to the component triaryl phosphate flame retardants triphenyl phosphate (TPP) and isopropylated triphenyl phosphate (ITP) and the prototypical environmental PPARγ ligands tributyltin (TBT) and triphenyltin (TPT) [23]. Cytotoxicity was investigated as a potential cause of decreased reporter gene activity at high concentrations. Increasing concentrations of FM 550, TPP or ITP caused cell death in reporter CHO cells and in human brain tumor cells where effects were blocked by inhibitors of caspase 3 dependent apoptosis. However, in reporter cells, normalization of PPARγ activity to viable cell number did not account fully for the decrease in PPARγ activity, which suggests that the observed nonmonotonic C/R relationship resulted from cytotoxicity and other undefined mechanisms. Ligand-induced receptor desensitization or down regulation was not a likely contributing factor since the more efficacious PPARγ agonist rosiglitizone increased PPARγ activity in a concentration dependent manner without high-concentration loss of activity [23].

Nonmonotonic C/R relationships arising from receptor selectivity of ligands

Due to the varying affinity that NR ligands such as BPA have for different NRs, ligand selectivity can also contribute to nonmonotonic C/R relationships. [2, 11–13]. The receptor selectivity of BPA may explain the observation that developmental BPA exposure causes increased locomotor activity of zebrafish larvae at doses from 10 to 100 nM, but has no

effect at higher doses. The observed increases in locomotor activity are likely due to ERα or ERβ dependent effects as 100 nM 17β-estradiol (E2) also increased locomotor activity of the zebrafish larvae. However, the underlying mechanism responsible for the nonmonotonic dose response relationship (NMDR) was not investigated [24]. One possible explanation for this observed NMDR is that BPA can act as an ER agonist, and also an AR antagonist at high concentrations [25]. This possibility is supported by the observation that the flame retardant DE-71, which has defined anti-androgen activity, also decreases locomotor activity in zebrafish [26,27]. It is possible that ER activation at low doses of BPA (10 and 100 nM) were responsible for increased locomotor activity, while inhibition of the AR counteracted this effect at the higher doses.

Nonmonotonic C/R relationships resulting from nuclear receptor effects on multiple endpoints

Nonmonotonic C/R relationships can also result when a single NR affects multiple oppositional signaling pathways. This complexity may be due to the observation that a single NR can mediate different cellular responses due to the cellular localization of the receptor. For example, ERs are known to localize to the nucleus, cytoplasm, mitochondria and the plasma membrane and ERs can have differential effects on proliferation, cell survival, Ca^{2+} handling and other endpoints due to the cellular localization of the activated receptor [3,6,28–30]. The response of cerebellar granule cell precursors (GCPs) to 17β-estradiol (E2) serves as an example of how differentially localized NRs can oppose one another to result in a complex C/R relationship [31–33]. Exposure of cultured GCPs to E2 rapidly increased ERK phosphorylation through a membrane associated ER at 10 to 100 pM, but has no effect at 1–100 nM and increased ERK phosphorylation at 1 μM. Similar effects are also observed *in vivo* [31,33]. Further investigation into the mechanism responsible for this nonmonotonic C/R relationship revealed that low E2 (100 pM) also activated a cytoplasmic ER to induce the activation of protein phosphatase 2a (PP2a) which opposed the effects of the membrane associated ER by de-phosphorylating MEK [32]. The inhibitory phosphatase activity was considered responsible for the observed temporal and nonmonotonic C/R effects of E2 on rapid ERK-signaling these neurons.

The response of LNCaP cells treated with the synthetic androgen R1881 is another example of a complex C/R relationship that arises due to multiple endpoints being affected by a single NR. Androgen deprived LNCaP cells are resistant to TNF-related apoptosis-inducing ligand (TRAIL) induced cell death due to down regulation of death receptor 5 (DR5) [34]. It was observed that 0.1 nM R1881 induced DR5 expression and sensitized LNCaP cells to TRAIL induced cell death. However, higher concentrations of R1881 were not as effective in sensitizing the LNCaP cells to TRAIL induced cell death. It was suggested that the well-known upregulation of pro-survival proteins in response to AR activation in LNCaP cells may be responsible for the observed nonmonotonic C/R relationship [34].

A more clearly defined mechanism was determined for an observed nonmonotonic C/R relationship in response to BPA treatment in rat cardiomyocytes where 1 pM to 10 nM BPA increased fractional shortening and 1 μM BPA decreased this effect [35]. It was determined that activation of ERβ by BPA resulted in a concentration dependent increase in Ca^{2+} release

from the sarcoplasmic reticulum, and BPA inhibited the L-type calcium channel current at concentrations at or above 1 μM [35]. The ERβ dependent increase in SR Ca^{2+} release in response to low concentrations of BPA resulted in increased cytoplasmic Ca^{2+} and a corresponding increase in cardiomyocyte contractility. However, the ERβ dependent inhibition of the L-type calcium channel current in response to high concentrations of BPA resulted in decreased cytoplasmic Ca^{2+} and a corresponding decrease in cardiomyocyte contractility [35,36]. This suggests that the observed nonmonotonic C/R relationship for BPA was due to the opposition of these two endpoints which were both dependent of ERβ. These studies thus demonstrate that NRs can have effects on multiple opposing endpoints that result in the generation of nonmonotonic C/R relationships.

Secondary NR binding site mediated C/R complexity

The observation of low affinity secondary binding sites on NRs suggests that a biologically plausible molecular mechanism may contribute to C/R complexity. While NR ligands modulate NR activity by binding in the ligand binding pocket (LBP) within the ligand binding domain (LBD) of the receptor, low affinity secondary binding sites that may affect receptor function have also been observed [37–39]. The observation that several of these secondary binding sites are in the co-regulator groove and could interfere with co-regulator binding suggests that these sites are functionally significant [38–40]. Furthermore, since NR ligands bind at both the primary site in the LBP and the secondary low affinity binding site, nonmonotonic C/R relationships can arise if the effects of binding at the different sites are in opposition (Fig. 2) [38,39]. This molecular mechanism was first supported by the observation that the binding capacity of the ER for tamoxifen was twice that of E2, suggesting that tamoxifen bound within the LBP and at an unknown secondary site [41]. A two binding site model of anti-estrogen action was then proposed in which binding to the higher affinity LBP site mediated the agonist actions of selective ER modulators (SERMS) such as tamoxifen, while binding at a low affinity secondary site mediated the antagonist actions of the compound [38]. In support of the two-site model, a low affinity secondary binding site for hydroxytamoxifen within the co-activator binding groove of ERβ was identified using x-ray crystallography [42]. This observation revealed that ligand occupancy of the secondary site could interfere with co-activator recruitment and affect ER transactivational activity.

A secondary ligand binding site has also been observed in the TR and PPARγ [39,40]. X-ray crystallography was used to characterize the binding of the PPARγ partial agonist ajulemic acid. While ajulemic acid was observed to bind the primary binding site within the LBP, it also bound to a secondary site on the surface of the receptor within the co-regulator groove. Binding at the secondary site may regulate receptor function by affecting co-regulator recruitment, but the functional significance of the secondary binding site was not investigated [40]. X-ray crystallography was also used to characterize the binding of the endogenous TR ligands T_3 and T_4. It was observed that both hormones not only bind the LBP site, but also bind at a secondary site on the surface of the LBD. Site directed mutagenesis and transactivation assays suggested that hormone binding at the secondary site was important for TR function [39]. Additionally, the binding affinity of T_3 ($K_d \approx 90.9$ nM) and T_4 ($K_d \approx 0.04$ nM) at the secondary binding site was shown to differ from that of T_3

($K_d \approx 0.06$ nM) and T_4 ($K_d \approx 2$ nM) at the primary site [39,43]. If the effects of binding at the primary and secondary sites are oppositional, the differing affinity may contribute to a complex C/R relationship. Additionally, while T_3 is thought to be the primary TR ligand *in vivo*, the high affinity binding of T_4 to the secondary site suggests that TR function may also be regulated by T_4 [39,43].

The existence of secondary binding sites on NRs suggests that NR ligands could cause complex C/R relationships through the binding at two sites on a NR with differing affinity that have opposing actions on receptor function. This possibility is supported at the molecular level by high-resolution characterization of secondary binding sites on several NRs. The functional importance of these sites are supported by their location in the co-regulator binding groove, and by the fact that mutations within the secondary site can affect receptor transactivational activity [38–42]. These studies demonstrate that it is biologically plausible that differential binding of a NR ligand to both primary and secondary binding sites contributes to C/R complexity (Fig. 2).

Conclusions

Cytotoxicity is likely the most common mode of action responsible for complex C/R relationships but additional factors can also contribute to this phenomenon. The contributing mechanisms may include ligand selectivity, receptor downregulation/desensitization, receptor and cofactor expression, receptor competition, endocrine negative feedback loops, the activation of multiple pathways by a NR, and secondary binding sites on NRs [2,38,39]. Support for these mechanisms include the fact that EDCs such as BPA bind and affect the activation of several NRs with varying affinity and that low affinity secondary binding sites exist on NRs that affect the transactivational activity of NRs [10–13, 38–42]. Overall, the studies reviewed here demonstrate that several modes of action for C/R complexity are biologically plausible and are supported at the whole animal, cellular and molecular levels. The implications of nuclear receptor mediated complexity should be considered when interpreting results from toxicological studies to accurately gauge potential hazards that may not be predicted by extrapolating from the results of high dose studies.

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Abbreviations

AR	androgen receptor
BPA	bisphenol A
C/R	concentration response
DR	death receptor
E2	17β-estradiol

EDC	endocrine disrupting chemical
ER	estrogen receptor
EC50	concentration of half maximal effect
FM 550	Firemaster® 550
GCP	granule cell precursor
HUVEC	human umbilical vein endothelial cells
ITP	isopropylated triphenyl phosphate
IC_{50}	concentration of half maximal inhibition
LBD	ligand binding domain
LBP	ligand binding pocket
NMDR	nonmonotonic dose response
NR	nuclear receptor
PP2a	protein phosphatase 2a
PPAR	peroxisome proliferator-activated receptor
SERM	selective estrogen receptor modulator
T3	Triiodothyronine
T4	thyroxine
TR	thyroid hormone receptor
TRAIL	TNF-related apoptosis-inducing ligand
TBT	tributyltin
TPP	triphenyl phosphate
TPT	triphenyltin

References

1. Kohn MC, Melnick RL. Biochemical origins of the non-monotonic receptor-mediated dose-response. J Mol Endocrinol. 2002; 29:113–23. [PubMed: 12200233]
2. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee D-H, Shioda T, Soto AM, vom Saal FS, Welshons WV, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012; 33:378–455. [PubMed: 22419778]
3. Kininis M, Kraus WL. A global view of transcriptional regulation by nuclear receptors: gene expression, factor localization, and DNA sequence analysis. Nucl Recept Signal. 2008; 6:e005. [PubMed: 18301785]
4. Zhang D, Trudeau VL. Integration of membrane and nuclear estrogen receptor signaling. Comp Biochem Physiol A Mol Integr Physiol. 2006; 144:306–15. [PubMed: 16516516]
5. Brent GA. Mechanisms of thyroid hormone action. J Clin Invest. 2012; 122:3035–43. [PubMed: 22945636]
6. Hammes SR, Levin ER. Minireview: Recent advances in extranuclear steroid receptor actions. Endocrinology. 2011; 152:4489–95. [PubMed: 22028449]

7. Kuiper GG, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, Gustafsson Ja. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 1997; 138:863–70. [PubMed: 9048584]

8. Safe S, Kim K, Kim K. Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. Journal of molecular endocrinology. 2008; 41:263–275. [PubMed: 18772268]

9. Wu F, Khan S, Wu Q, Barhoumi R, Burghardt R, Safe S. Ligand structure-dependent activation of estrogen receptor alpha/sp by estrogens and xenoestrogens. The Journal of steroid biochemistry and molecular biology. 2008; 110:104–115. [PubMed: 18400491]

10. Stice JP, Knowlton AA. Estrogen, NFkappaB, and the heat shock response. Molecular medicine (Cambridge, Mass). 2008; 14:517–527.

11. Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. J Endocrinol. 1998; 158:327–39. [PubMed: 9846162]

12. Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, Hataya Y, Shimatsu A, Kuzuya H, Nakao K. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab. 2002; 87:5185–90. [PubMed: 12414890]

13. Paris F, Balaguer P, Térouanne B, Servant N, Lacoste C, Cravedi J-P, Nicolas J, Sultan C. Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit alpha and beta estrogen activities and antiandrogen activity in reporter cell lines. Mol Cell Endocrinol. 2002; 193:43–9. [PubMed: 12161000]

14. Ellem SJ, Risbridger GP. The dual, opposing roles of estrogen in the prostate. Ann N Y Acad Sci. 2009; 1155:174–86. [PubMed: 19250203]

15. Chao W, Xuexin Z, Jun S, Ming C, Hua J, Li G, Tan C, Xu W. Effects of resveratrol on cell growth and prolactin synthesis in GH3 cells. Exp Ther Med. 2014; 7:923–928. [PubMed: 24669252]

16. Choi EJ, Kim G-H. Antiproliferative activity of daidzein and genistein may be related to ERα/c-erbB-2 expression in human breast cancer cells. Mol Med Rep. 2013; 7:781–4. [PubMed: 23337939]

17. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem. 1987; 262:5592–5. [PubMed: 3106339]

18. Thomas GW, Rael LT, Bar-Or R, Mains CW, Slone DS, Boyd SR, Bar-Or D. Biphasic effect of danazol on human vascular endothelial cell permeability and f-actin cytoskeleton dynamics. Biochem Biophys Res Commun. 2012; 421:707–12. [PubMed: 22542943]

•19. Mahmoud AM, Zhu T, Parray A, Siddique HR, Yang W, Saleem M, Bosland MC. Differential effects of genistein on prostate cancer cells depend on mutational status of the androgen receptor. PLoS One. 2013; 8:e78479. Using prostate cancer cell lines with WT and mutant ARs, the authors demonstrate that genistein dose dependently decreases cell proliferation unless the mutant AR is expressed. When the mutant AR was expressed, genistein affected proliferation nonmonotonically by activating the mutant AR at low concentration but inhibiting proliferation at high concentrations similarly to what occurs in cells expressing the WT AR. [PubMed: 24167630]

20. Wetherill YB, Petre CE, Monk KR, Puga A, Knudsen KE. The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostatic adenocarcinoma cells. Mol Cancer Ther. 2002; 1:515–24. [PubMed: 12479269]

21. Wetherill YB, Fisher NL, Staubach A, Danielsen M, de Vere White RW, Knudsen KE. Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status. Cancer Res. 2005; 65:54–65. [PubMed: 15665279]

22. Thomas GW, Rael LT, Shimonkevitz R, Curtis CG, Bar-Or R, Bar-Or D. Effects of danazol on endothelial cell function and angiogenesis. Fertil Steril. 2007; 88:1065–70. [PubMed: 17382938]

•23. Belcher SM, Cookman CJ, Patisaul HB, Stapleton HM. In vitro assessment of human nuclear hormone receptor activity and cytotoxicity of the flame retardant mixture FM 550 and its triarylphosphate and brominated components. Toxicol Lett. 2014; 228(2):93–102. Using a luciferase reporter system, the authors demonstrate that the flame retardant mixture FM 550 and

its triarylphosphate components nonmonotonically increased PPARγ dependent expression. Importantly, while cytotoxicity was increased by both FM 550 and its triarylphosphate components, the cytotoxicity did not fully account for the nonmonotonic effect. [PubMed: 24786373]

•24. Saili KS, Corvi MM, Weber DN, Patel AU, Das SR, Przybyla J, Anderson Ka, Tanguay RL. Neurodevelopmental low-dose bisphenol A exposure leads to early life-stage hyperactivity and learning deficits in adult zebrafish. Toxicology. 2012; 291:83–92. Using a larval zebrafish as a model, the authors demonstrate that BPA exposure results in hyperactivity. The hyperactivity is not observed at high doses, but the mechanism for the NMDR is not investigated. Due to the known selectivity of BPA for NRs, the result may be due to ligand selectivity. [PubMed: 22108044]

25. Teng C, Goodwin B, Shockley K, Xia M, Huang R, Norris J, Merrick BA, Jetten AM, Austin CP, Tice RR. Bisphenol A affects androgen receptor function via multiple mechanisms. Chem Biol Interact. 2013; 203:556–64. [PubMed: 23562765]

26. Chen L, Huang C, Hu C, Yu K, Yang L, Zhou B. Acute exposure to DE-71: effects on locomotor behavior and developmental neurotoxicity in zebrafish larvae. Environ Toxicol Chem. 2012; 31:2338–44. [PubMed: 22833361]

27. Stoker TE, Cooper RL, Lambright CS, Wilson VS, Furr J, Gray LE. In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. Toxicol Appl Pharmacol. 2005; 207:78–88. [PubMed: 16005038]

28. Pedram A, Razandi M, Wallace DC, Levin ER. Functional estrogen receptors in the mitochondria of breast cancer cells. Mol Biol Cell. 2006; 17:2125–37. [PubMed: 16495339]

29. Razandi M, Alton G, Pedram A, Ghonshani S, Webb P, Levin ER. Identification of a structural determinant necessary for the localization and function of estrogen receptor alpha at the plasma membrane. Mol Cell Biol. 2003; 23:1633–46. [PubMed: 12588983]

30. Belcher SM, Chen Y, Yan S, Wang H-S. Rapid estrogen receptor-mediated mechanisms determine the sexually dimorphic sensitivity of ventricular myocytes to 17β-estradiol and the environmental endocrine disruptor bisphenol A. Endocrinology. 2012; 153:712–20. [PubMed: 22166976]

31. Zsarnovszky A, Le HH, Wang HS, Belcher SM. Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology. 2005; 146:5388–5396. [PubMed: 16123166]

32. Wong JK, Le HH, Zsarnovszky A, Belcher SM. Estrogens and ICI182,780 (Faslodex) modulate mitosis and cell death in immature cerebellar neurons via rapid activation of p44/p42 mitogen-activated protein kinase. J Neurosci. 2003; 23:4984–4995. [PubMed: 12832521]

33. Belcher SM, Le HH, Spurling L, Wong JK. Rapid estrogenic regulation of extracellular signal-regulated kinase 1/2 signaling in cerebellar granule cells involves a G protein-and protein kinase A-dependent mechanism and intracellular activation of protein phosphatase 2A. Endocrinology. 2005; 146:5397–5406. [PubMed: 16123167]

34. Wang D, Lu J, Tindall DJ. Androgens regulate TRAIL-induced cell death in prostate cancer cells via multiple mechanisms. Cancer Lett. 2013; 335:136–44. [PubMed: 23402817]

••35. Liang Q, Gao X, Chen Y, Hong K, Wang H-S. Cellular Mechanism of the Nonmonotonic Dose Response of Bisphenol A in Rat Cardiac Myocytes. Environ Health Perspect. 2014 Using rat cardiomyocytes, the authors demonstrate the BPA acts through ERβ to both increase Ca^{2+} release from the SR and decrease L-type Ca^{2+} channel current. The opposing effect of increased Ca^{2+} release from the SR and L-type Ca^{2+} channel inhibition on fractional shortening, combined with the differing potency of BPA at each endpoint resulted in a nonmonotonic concentration response relationship. 10.1289/ehp.1307491

36. Eisner D, Bode E, Venetucci L, Trafford A. Calcium flux balance in the heart. J Mol Cell Cardiol. 2013; 58:110–7. [PubMed: 23220128]

37. Jin L, Li Y. Structural and functional insights into nuclear receptor signaling. Adv Drug Deliv Rev. 2010; 62:1218–26. [PubMed: 20723571]

38. Jensen EV, Khan Sa. A two-site model for antiestrogen action. Mech Ageing Dev. 2004; 125:679–82. [PubMed: 15541763]

••39. Souza PCT, Puhl aC, Martínez L, Aparício R, Nascimento aS, Figueira aCM, Nguyen P, Webb P, Skaf MS, Polikarpov I. Identification of a new hormone-binding site on the surface of thyroid hormone receptor. Mol Endocrinol. 2014; 28:534–45. The authors use x-ray crytallography to charactize a secondary thyroid hormone binding site on the surface on the TR. The secondary site had a lower affinity for the hormones and mutations withing the secondary binding site affected receptor function. [PubMed: 24552590]

40. Hedden, a; Müller, V.; Jensen, EV. A new interpretation of antiestrogen action. Ann N Y Acad Sci. 1995; 761:109–20. [PubMed: 7625715]

41. Wang Y, Chirgadze NY, Briggs SL, Khan S, Jensen EV, Burris TP. A second binding site for hydroxytamoxifen within the coactivator-binding groove of estrogen receptor beta. Proc Natl Acad Sci U S A. 2006; 103:9908–11. [PubMed: 16782818]

42. Ambrosio ALB, Dias SMG, Polikarpov I, Zurier RB, Burstein SH, Garratt RC. Ajulemic acid, a synthetic nonpsychoactive cannabinoid acid, bound to the ligand binding domain of the human peroxisome proliferator-activated receptor gamma. J Biol Chem. 2007; 282:18625–33. [PubMed: 17462987]

43. Sandler B, Webb P, Apriletti JW, Huber BR, Togashi M, Cunha Lima ST, Juric S, Nilsson S, Wagner R, Fletterick RJ, et al. Thyroxine-thyroid hormone receptor interactions. J Biol Chem. 2004; 279:55801–8. [PubMed: 15466465]

Highlights

- Concentration response complexity arises in response to nuclear receptor ligands.
- Secondary binding sites may contribute to concentration response complexity.
- Concentration response complexity may arise due to the lack of ligand specificity.

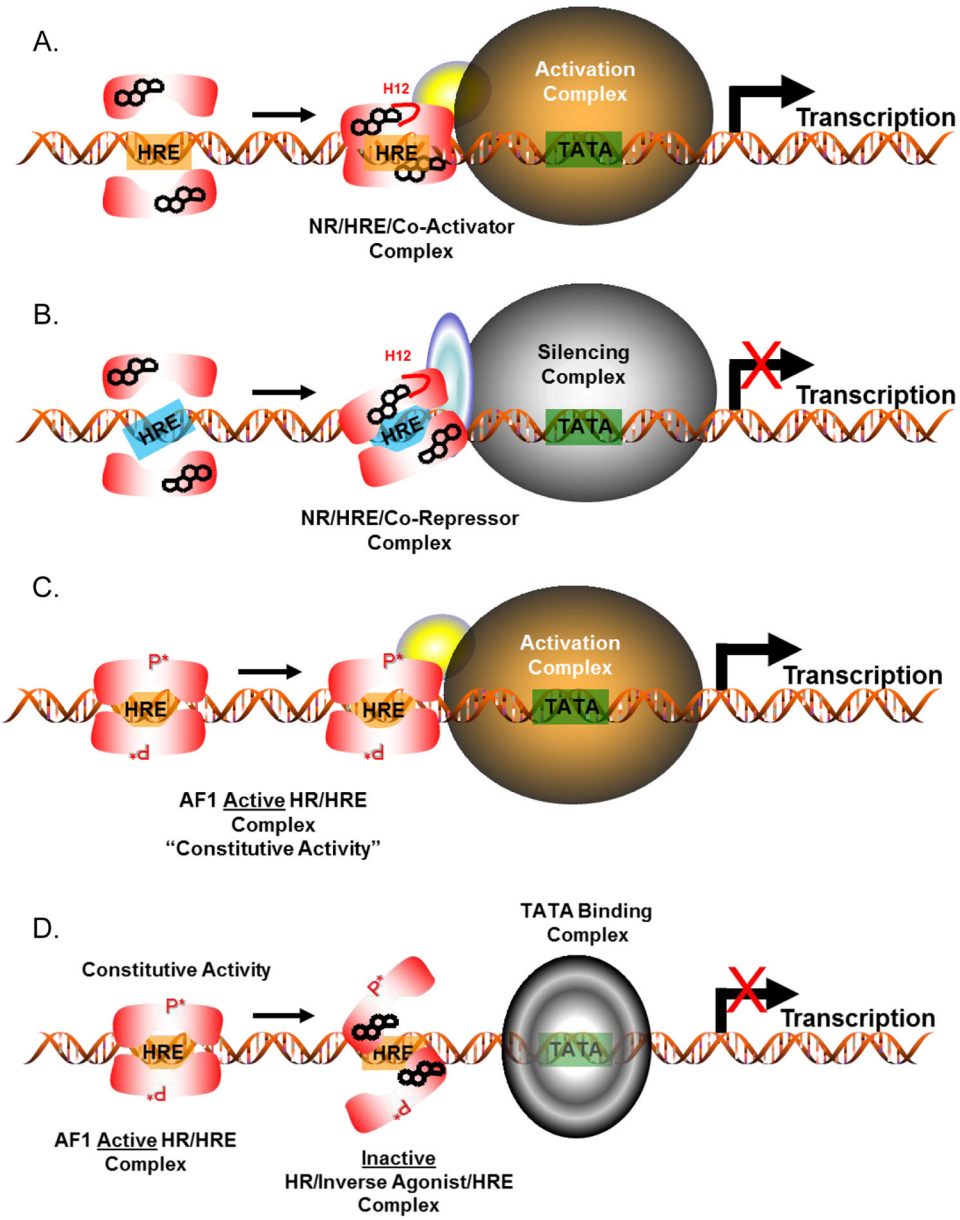

Figure 1. The multiple mechanisms of nuclear receptor mediated modulation of responsive gene transcription

Binding of ligand induces a conformational change of the NR that allows receptor dimerization, association with hormone responsive elements (HRE) in the promoters of responsive genes, and recruitment of **(A)** co-activators resulting in an increase in responsive gene expression, or **(B)** recruitment of co-repressors that repress gene expression. **(C)** Nuclear receptor activity can also be induced by receptor phosphorylation of the Activator Function 1 (AF1) domain resulting in ligand independent constitutive activity. **(D)** Binding of ligand may cause a conformational change of constitutively active or constitutively bound NR that counteracts the effects of a constitutively active receptor. Pharmacologically, such effects are equivalent to inverse-agonist effects.

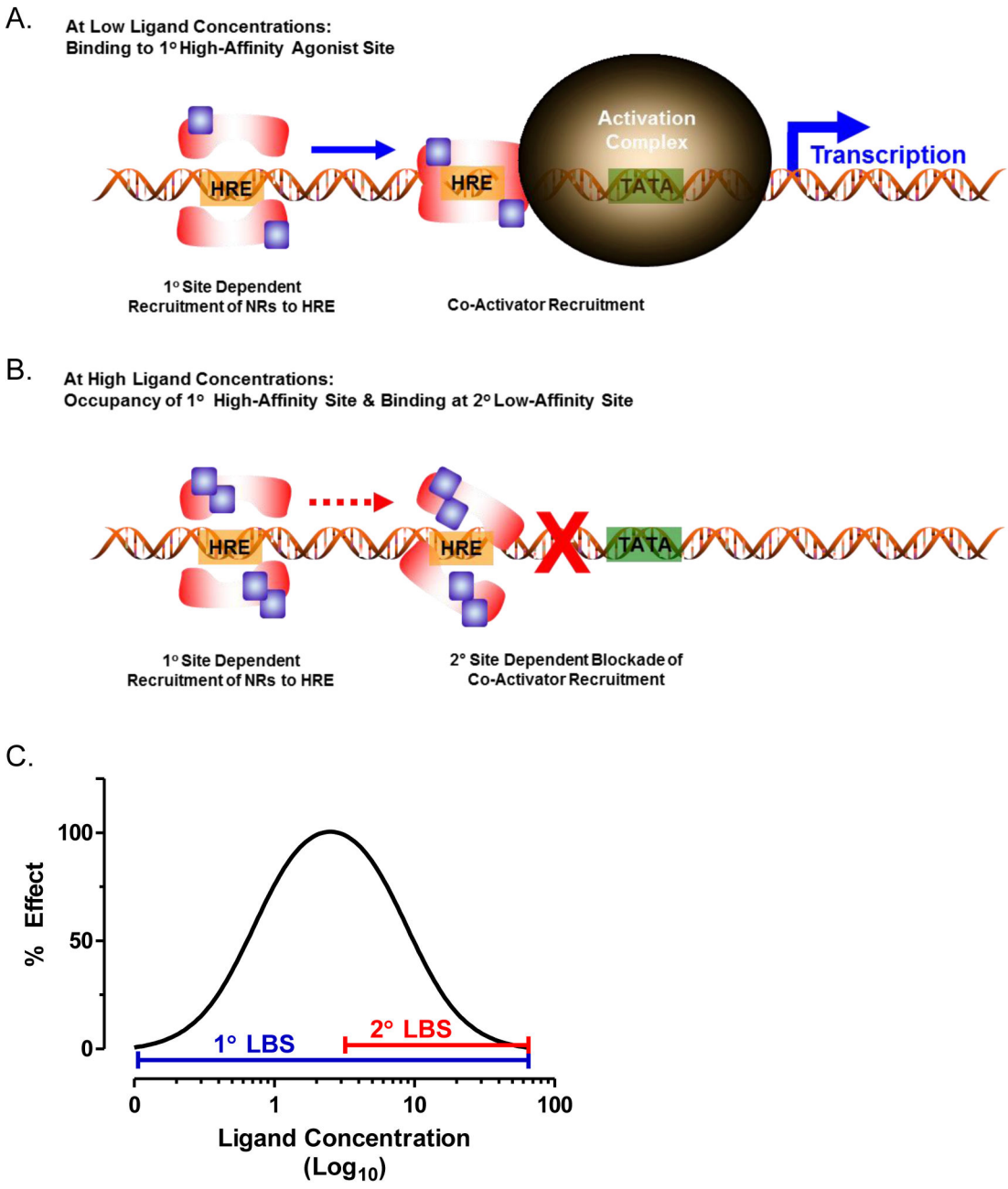

Figure 2. Two ligand binding site model contributing to complex C/R relationships in response to NR ligands

(A) Ligand (blue square) binding to the high affinity primary site results in receptor dimerization, binding to the hormone responsive element (HRE), and recruitment of co-activators to increase responsive gene transcription. **(B)** Ligand binding to the high affinity primary site induces recruitment of the NR to the HRE, and ligand binding to the low affinity secondary binding site within the co-activator groove blocks the recruitment of co-regulators, thereby preventing modulation of gene transcription. **(C)** Idealized concentration response curve based on the hypothetical binding of a single ligand to a high affinity

stimulatory ligand binding site and a low affinity inhibitory binding site. The concentration range of the agonist effects of the high affinity site to the C/R curve are indicated by a blue line and the impacts on the curve of the low affinity inhibitory site are indicated with a red line.