Environmental Estrogens: Can Two "Alrights" Make a Wrong?

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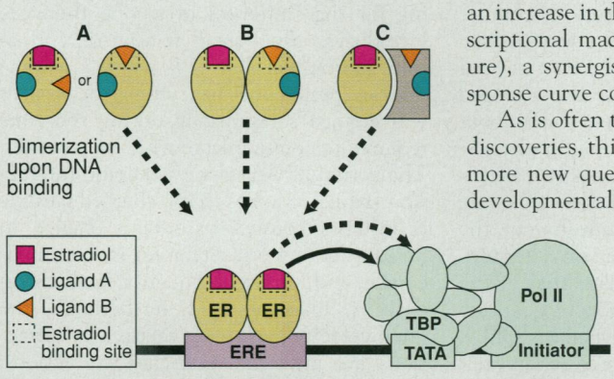
We all want to know whether substances in our environment are harmful to us and other animals. But answering this question is no easy task. A report in this issue of *Science* (*1*) admirably illustrates why. Two synthetic chemicals that are individually innocuous turn out to have effects like the female estrogenic hormones when they are mixed together.

Most developmental effects of estrogens are mediated by their binding to an intracellular steroid receptor protein, a member of a large superfamily. Before an estrogen binds, the receptor is associated with several nonreceptor proteins, such as the chaperone protein heat shock protein 90. Estrogens initiate the dissociation of nonreceptor proteins and the binding of homodimeric receptor-steroid complexes to palindromic hormone response elements in the DNA. These DNA-bound receptors regulate transcription of estrogen-responsive genes by interacting with the transcriptional machinery in a manner that can be modified by coactivators, repressors, and modulators.

Arnold and co-workers (*1*) have used yeast cells transformed with the human estrogen receptor (hER) to quantitate the estrogen activity of selected chemicals. They measured the ability of each chemical to mobilize hER and induce the expression of β-galactosidase (β-Gal) encoded by an introduced lacZ gene driven by two copies of an estrogen response element. As in other systems, pesticides such as dieldrin or endosulfan were required at about 100,000 times greater concentration than the natural estrogen 17β-estradiol to cause half-maximal induction of expression. Surprisingly, dieldrin plus endosulfan (or other compounds) afforded a synergistic response—high levels of β-Gal were induced with a potency more than 100 times that of either compound alone [new half-maximal concentration (EC_{50}) ≈ 100 nM (*2*)]. Combinations of three substances had little further increase in potency. Two hydroxylated polychlorinated biphenyls (PCBs) also acted synergistically to induce β-Gal expression, albeit to a lesser extent (fivefold).

Although yeast expression systems are useful for steroid studies, they differ in unexplained ways from mammalian cells (*2, 3*). Anti-estrogens, usually antagonists in mammalian cells, have substantial agonist activity in yeast (*4*), and different regions of the hER have independent transactivation activity in yeast and mammalian cells (*5*). So it is crucial that the authors have also demonstrated in human endometrial cells the same fivefold synergistic increase in estrogenic response by combinations of PCBs as seen in the yeast system. Thus, the synergistic estrogenic effects of these compounds are more than a laboratory idiosyncrasy of yeast cells.



How environmental estrogens might synergize. The normal homodimeric hER-estradiol complex binds to an estrogen response element (ERE) and interacts with the transcriptional machinery (solid arrow). Modified, and potentially synergistic, interactions could be accomplished by binding to ERE (dashed arrows) of (**A**) initially monomeric receptors with multiple ligands, (**B**) heterodimeric complexes of a single protein but with different ligands, or (**C**) a dimer of two different proteins.

Equally remarkable is that similar synergistic potency increases were seen for direct binding of combined compounds to recombinant hER. This parallel between enhanced estrogen binding affinity and receptor-regulated biological activity of the combined ligands suggests that the mechanistic basis of the synergism is at the hER. It may be time to resurrect the hypotheses of multiple binding sites on each receptor monomer (*6, 7*) (see the figure), despite the fact that the recent x-ray structures of the related retinoic acid and thyroid receptors show only one agonistic ligand per isolated steroid binding domain (*8*).

The synergistic binding of dieldrin and endosulfan to hER could competitively inhibit only about 60% of the estradiol binding, suggesting the presence of two binding sites for estradiol—only one of which could be occupied by dieldrin and endosulfan. This stoichiometry is difficult to explain if binding occurs to monomeric estrogen receptors (see figure, part A). However, estrogen receptor binding can be highly cooperative, thus involving receptor dimers (*9*). Therefore, the binding of estradiol to only one of the two sites might be competed by a synergistic binding of dieldrin and endosulfan to half of the dimer (part B). Alternatively, dieldrin and endosulfan could synergistically bind to a different molecule that competes with ER for homodimer formation (part C). Indeed, other classical steroid receptors have been shown to form heterodimers (*10*). Furthermore, estrogen receptor heterodimers could have different biological activities (*10, 11*) and possibly altered ligand binding properties (*11*). If any of these complexes caused an increase in the coupling efficiency of transcriptional machinery components (see figure), a synergistic left shift in the dose-response curve could ensue (*12*).

As is often the case with significant new discoveries, this paper by Arnold *et al.* poses more new questions than it answers. Are developmentally important, estrogen receptor-regulated genes synergistically induced by these new environmental estrogens? Can the various pesticides synergize in mammalian cells and, if so, why is the magnitude of synergism so much greater than for the PCBs? How should the risk of new chemicals be assessed? Are there multiple ligand binding sites on hER? Does the hER heterodimerize with nonreceptor proteins? How is a left shift in the dose-response curve effected? The pursuit may never end, but we are making progress.

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