Synergistic Action of 17-β estradiol and Bisphenol A in a Pituitary Cell Line

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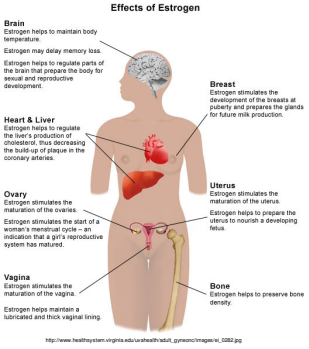
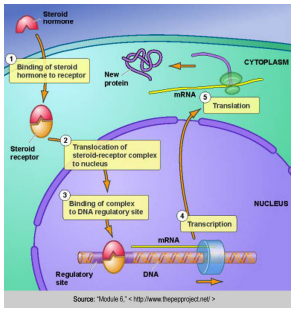
INTRODUCTION

The gonadal steroid 17 β-estradiol (E_2) is produced primarily by the ovaries and regulates gene regulation, growth and differentiation in a number of target tissues, including the pituitary gland, where E_2 regulates the production and/or release of hormones such as prolactin and luteinizing hormone.



Estrogen receptor alpha (ERα) is a ligand-inducible transcription factor that mediates the physiological effects of E_2. In a target cell, ERα occupancy by E_2 results in receptor activation and nuclear localization. Subsequent binding to a regulatory site (estrogen response element (ERE)) in a target gene allows genes to be activated, and ultimately, produces proteins are produced that elicit physiological effects



The actions of endogenous E_2 can be mimicked by environmental estrogens that are produced in plants (phytoestrogens) or produced commercially as synthetic compounds (xenoestrogens). These studies examine changes in gene transcription following co-administration of E_2 and a variety of E_2 mimics

MATERIALS AND METHODS

Cells

GH_3 cells (a rat pituitary cell line secreting both prolactin and growth hormone) were cultured in DMEM with growth serum; cells were maintained at 37ºC in 95%O_2/5%CO_2.

Gene Expression in Mammalian Cells

100 ng rat ERα and 200ng pGL3 reporter (ERE gene fused to firefly luciferase gene) were transiently transfected into GH_3 cells. For all studies, cells were treated for 24 hours with 10nM E_2, and/or the estrogen mimics bisphenol A (BA), genistein (G), daidzein (D), and kepone (K) at doses indicated in respective figures. In antagonist studies, the ERα antagonist ICI 182,780 was used at a concentration of 1μM. Cells were collected in 200 ul 1X Promega lysis buffer.

To measure transcriptional activity, 50ul luciferin was added to an equal volume of lysate and luciferase activity assessed using a Turner Biosystems 20/20n luminometer. Data are expressed as arbitrary light units (ALU)/mg protein.

Statistical Analysis.

Transfections were performed in triplicate. Data were analyzed by one way ANOVA and a Bonferroni post-hoc test using GraphPad Prism software.

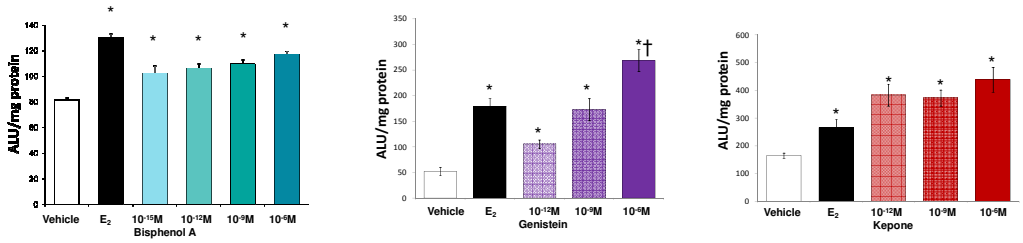


Figure 1. Bisphenol A (BA, xenoestrogen), genistein (G, a phytoestrogen), and kepone (K, a xenoestrogen) stimulate transcriptional activity of the pGL3 reporter in a dose-related fashion. Cells were transfected with ERα and the pGL3 reporters. Administration of physiological doses of BA, G, and K significantly increased transcriptional activity (as compared to vehicle treated controls). There was a positive correlation between increasing concentrations and transcriptional activity *Significantly different from vehicle controls, p<.01 or p<.05., † significantly different from 10^{-9}M and 10^{-12}M G

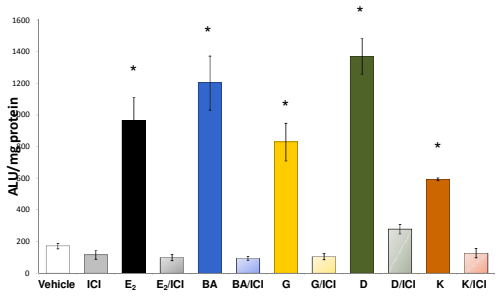


Figure 2. Stimulatory effects on transcriptional activity in GH_3 cells are mediated by ERα. Cells were transfected with ERα and pGL3 reporter, then stimulated with E_2 (10^{-8}M) , or a panel of xeno/phytoestrogens (10^{-6}M) in the absence and presence of the pure ERα antiestrogen, ICI 182,780. Transcriptional effects of the estrogenic compounds were ameliorated in the presence of ICI 182,780, indicating that these effects are receptor mediated. *Significantly different from vehicle controls, p<.001

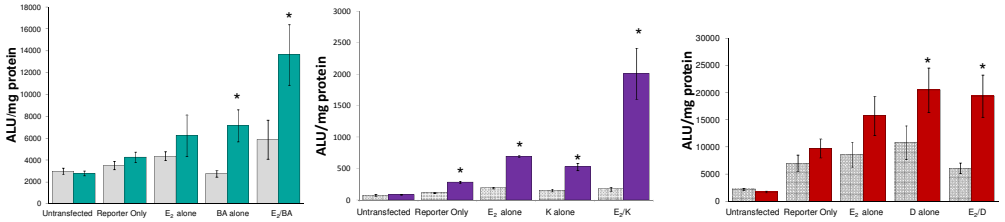


Figure 3. The xenoestrogens kepone acts synergistically with E_2 to stimulate transcriptional activity of the pGL3 reporter. GH_3 cells were transfected with ERα and pGL3 reporter. Transfected cells were treated with vehicle, E_2 alone, xenoestrogen alone, or E_2 plus xenoestrogen. White bars represent vehicle treated cells in each group.*Significantly different from vehicle controls, p<.01

SUMMARY AND CONCLUSIONS

The steroid hormone E_2 targets the pituitary gland and the brain where it regulates, among other things, the growth and release of reproductive hormones. It is clear that the presence of one hormone may be required for another hormone to have its designated physiological effect, as is the case in priming. Moreover two hormones may act at their respective receptors to achieve a controlled physiological response. The sensitivity of the pituitary gland to E_2 and the ability of non-steroidal compounds to bind/activate the ERE have led us to examine interactions between estrogenic compounds and their subsequent effect on gene transcription on a model promoter (pGL3).

Cells exhibited moderate (1.5 fold) to robust (7 fold) dose-related transcriptional responses to single treatments of E_2, xenoestrogens, or phytoestrogens. As indicated in Figure 1, the stimulating concentrations of xeno/phytoestrogens were well within the physiological range that most endogenous hormones circulate. Antagonist studies (Figure 2) indicate that the panel of xeno/phytoestrogens used in the studies utilize the same receptor as E_2, Thus, as we consider the greater than additive (E_2 and BA) or synergistic (E_2 and kepone) effects on transcription that are observed with are co-administered, it is possible that results are due to differential recruitment of cofactors in the nucleus during gene activation. Further studies will examine this hypothesis.

ACKNOWLEGMENTS

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