Environmental Estrogens Stimulate Gene Transcription in the Prolactin Promoter

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INTRODUCTION
17 β-estradiol (E2) is produced primarily by the ovaries and governs gene expression in a number of target tissues, including the brain and pituitary. In the pituitary, particularly, the role of estrogen extends to stimulating reproductive hormone secretion. When estrogen receptor alpha (ERα) is a ligand-inducible transcription factor that mediates the physiological effects of E2. Within a target cell, occupancy of ERs by E2 results in receptor activation and nuclear localization. Subsequent binding to an estrogen response element (ERE) in a target gene allows recruitment of various coregulator proteins which enhance or suppress gene expression.

MATERIALS AND METHODS
Cells
GH3 cells (rat pituitary cell line expressing endogenous ERα) were cultured in DMEM supplemented with growth serum and maintained at 37°C in 95% O2/5% CO2.

Gene Expression in Mammalian Cells
All cell lines were transiently transfected using Genejuice transfection reagent. To determine dose response relationships, 250 ng pGL3 reporter (ERE gene fused to firefly luciferase gene) were transfected into GH3 cells and treated with increasing concentrations of the xenoestrogen bisphenol A and the phytoestrogen daidzein. Alternatively, cells were transfected with 100 ng prolactin promoter and treated with increasing concentrations of the xenoestrogen bisphenol A and the phytoestrogen. To demonstrate that the effects of environmental estrogens were receptor-mediated, treatments were also administered in the presence of ICI 182 780, a pure ERα antagonist.

To examine the role of the transcription factors, Pit-1, cells were transfected with prolactin promoter and Pit-1, then treated with environmental estrogens.

For each set of experiments, period of treatment was 24 hours. Cells were then collected in 200 ul 1X Promega lysis buffer. 50ul luciferin was added to an equal volume of lysate and luciferase activity assessed using a Turner Biotek 20/20l luminometer. Data are expressed as arbitrary light units (ALU) per 50 μl lysate or as 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. Environmental estrogens stimulate transcription in a dose related manner. GH3 cells were transfected with the 250 ng pGL3 model promoter (Panel A) or 250 ng of the physiologically complex PRL promoter (Panel B). Cells were treated for 24 hours with BPA (solid grey bars) or D (shadowed bars) at the doses indicated in the figure. Cells were collected, lysed and subjected to luciferase assay. Data are expressed as arbitrary light units per microgram of protein. Bars represent mean ± SEM for 4 experiments. For purposes of comparison, response of transfected cells to E2 is also shown (black bar). * significantly different from vehicle treated control. P<0.05. † significant difference in respective responses of EEs. P<0.05.

Table: Summary of transcriptional activity in both promoter constructs were stimulated by EEs in a dose related fashion. These stimulatory effects are mediated via the ER, since ICI (which competitively binds to estrogen to the ER) is observed to abolish these effects. Relative to the potency of E2, BPA, G and D proved to be more effective at stimulating transcription in the PRL promoter. In our experiments, the cotransfection of Pit-1 resulted in a moderate increase in EE-induced transcription. Thus, occupancy of the ER by EEs may elicit responses in the PRL promoter that require Pit-1 for full transcriptional activity. Collectively, these data indicate that EEs indeed behave similarly as E2 in the stimulation of the prolactin promoter with regard to mechanism but not magnitude. These data in these studies are significant because they demonstrate the effects of EEs on a pituitary specific gene promoter that is not regulated by a palindromic ERE, but multiple promoter elements.

REFERENCES

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