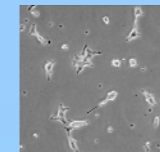




Differential Effects of Environmental Chemicals and Selected Pharmaceuticals on Aromatase Activity and Hormone Concentrations

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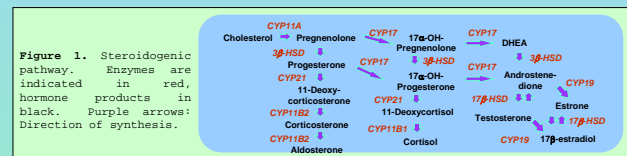


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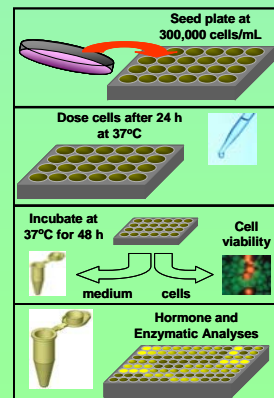
Introduction

Studies have suggested links between the exposure to natural and man-made substances in the environment and alterations in endocrine and reproductive systems in wildlife and humans. One process that is currently discussed as an important and relevant target for endocrine disrupting chemicals is the synthesis of sex steroids, specifically the production of 17 β -estradiol, by the enzyme aromatase. As a consequence, a number of assays have been developed to test for potential effects of chemicals on aromatase as a predictor for subsequent alterations of hormone homeostasis. Most of these assays, however, only measure a specific endpoint such as aromatase gene expression or enzyme activity, and it is unclear whether the observed changes are truly predictive of effects at the hormone level. Therefore, with this study we wished to investigate in parallel the effects of a number of known inducers and inhibitors of steroidogenic pathways on both aromatase enzyme activity (direct and indirect changes) and sex steroid concentrations. This was done using an *in vitro* screening assay developed to measure alterations in hormone production, the H295R cell steroidogenesis assay (1). Previous studies have indicated that this cell line was useful in screening for effects on gene expression of steroidogenic enzymes, aromatase enzyme activity and steroid hormones (1, 2, 3).



Methods

- Nine chemicals, including two selective aromatase inhibitors (fadrozole and aminoglutethimide), one fungicide (prochloraz), one herbicide (atrazine) and one general inducer of steroidogenesis (forskolin) were tested in the H295R steroidogenesis assay.
- Aromatase activity was measured using a tritium release assay (4). Three separate measurements for Aromatase activity was conducted involving seven different chemicals.
 1. Indirect measurement: Aromatase activity was measured after exposure to a chemical for 48 hours. The chemical was not added to tritium release assay.
 2. Direct measurement: Chemical was added directly to the tritium release assay with no pre-exposure of cells.
 3. Combined measurement: Aromatase activity was measured after chemical exposure for 48 hours and chemical was added directly to the tritium release assay.



H295R Steroidogenesis Assay (5)

1. Seed at a density of 300,000 cells/mL into 24 well plates, and incubate at 37°C and 5% CO₂ for 24h
2. Replace medium and dose cells
3. Incubate at 37°C and 5% CO₂ for 48h
4. Remove medium from cells and extract with ether
5. Conduct cell viability assay immediately after removal of medium (MTT (6) and live/dead assays)
6. Measure hormone concentrations in medium extract

Objectives

1. Identify effects of nine known or suspected endocrine disrupting chemicals on sex steroid production and aromatase enzyme activity in H295R cells.
2. Distinguish between direct and indirect acting compounds by means of differential aromatase activity analysis.
3. Evaluate the potential of using differential measurements of aromatase enzyme activity to predict effects on the homeostasis of T and E2.

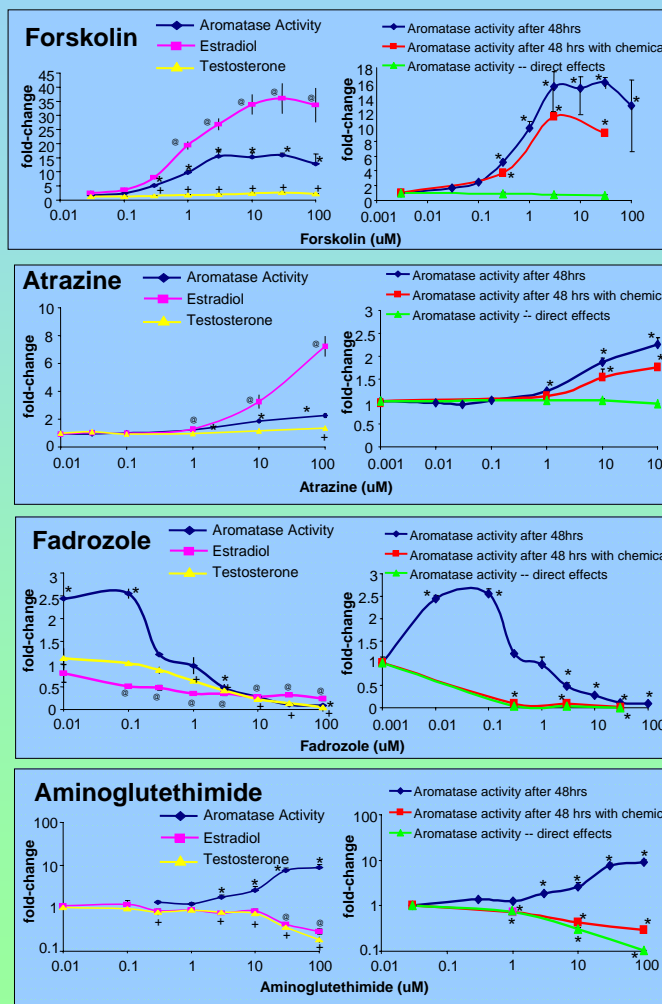


Figure 2. Relative change of testosterone, estradiol and aromatase activity of H295R cells exposed to Atrazine, Forskolin and Fadrozole. Symbols indicate significant differences from controls (* = Aromatase activity; + = Testosterone; @ = Estradiol)

Chemical	Testosterone	Estradiol	Aromatase Activity	Direct Aromatase Activity (Chemical added only to the assay)	Direct Aromatase Activity (Pre-exposed + chemical added to the assay)
Atrazine	↑	↑↑↑	↑↑	No change	↑
Forskolin	↑↑	↑↑↑	↑↑↑	No change	↑↑↑
Fadrozole	↓↓↓	↓↓	↑ then ↓↓↓	↓↓↓	↓↓↓
Prochloraz	↓↓↓	↓↓	↑↑ then ↓	↓↓↓	↓↓↓
Aminoglutethimide	↓↓↓	↓↓	↑↑↑	↓↓↓	↓↓
Ketoconazole	↓↓↓	↓↓	↑↑↑	↓	↑↑
Prometon	No Change	↑↑↑	↑↑	↓	No change
Trenbolone	↑↑↑	↑↑↑	No change	NA	NA
Trilostane	↑↑↑	↑↑↑	↓↓	NA	NA

Figure 3. Changes in the production of estradiol and testosterone, and activity of aromatase in H295R cells after exposure to all 9 chemicals studied. Magnitude of relative changes compared to solvent controls is represented by increasing number of arrows: One arrow: 1- to 2-fold change; Two arrows; 2- to 5-fold change; Three arrows: >5-fold change. Downward arrows indicate suppression; Upward arrows indicate increase. Only significant changes are listed (p<0.05).

Conclusion

- Changes in aromatase activity in response to exposure with forskolin and fadrozole were predictive of changes in estradiol production.
- All general inducers did not directly affect aromatase activity (i.e. Forskolin and Atrazine).
- Changes observed at the level of aromatase activity when either measured in the indirect or direct aromatase activity experiments were not always predictive of effects on estradiol and testosterone concentrations.
- However, integration of all endpoints measured in this study (testosterone, estradiol, direct, indirect and combined aromatase activity) provided a better insight into the potential mode of chemical interaction with the steroidogenic pathway investigated here..
- For compounds that do not directly compete with aromatase, the concentration of estradiol is likely to also be affected by upstream events as well as by other feedback mechanisms.
- Overall, measurement of aromatase activity and estradiol production can provide some insight into the mechanism of action of a chemical on endocrine and reproductive systems. However, further studies are needed to more specifically investigate whether a chemical is active at the gene expression level, protein level or is part of a feedback loop.

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