Concepts of Endocrinology: Issues Relevant to Endocrine Disruptor Screening

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OBJECTIVES OF PRESENTATION

• To provide a basic overview of some of the principles of endocrinology.
• To discuss specific aspects of endocrinology relevant to the assessment of endocrine disruptors (ED) with particular emphasis on issues that may
  – currently be overlooked or underestimated;
  – complicate the results and/or interpretation of ED screening assays or experiments.
Endocrine System: A mode of communication

• Endocrine gland
  – A ductless gland whose secretions are released into the extracellular fluid for action at a remote site.

• Hormone (Gr. “to excite or arouse”)
  – The chemical messenger secreted by an endocrine gland.
Hormones Synthesized and Secreted by Traditional Endocrine Glands
(protein/peptide, modified amino acid, steroid)

<table>
<thead>
<tr>
<th>Gland</th>
<th>Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>releasing and inhibiting hormones: GnRH, TRH, CRH, somatostatin, PIH (dopamine)</td>
</tr>
<tr>
<td>Pituitary</td>
<td>anterior - growth hormone, prolactin, ACTH, TSH, FSH, LH posterior - ADH, oxytocin</td>
</tr>
<tr>
<td>Thyroid</td>
<td>thyroxine (T4), triiodothyronine (T3), calcitonin</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>parathyroid hormone (PTH)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>insulin, glucagon, somatostatin</td>
</tr>
<tr>
<td>Adrenal</td>
<td>cortex - cortisol, aldosterone, and DHEA medulla - epinephrine, norepinephrine</td>
</tr>
<tr>
<td>Ovary</td>
<td>estradiol-17β, progesterone, inhibin</td>
</tr>
<tr>
<td>Testes</td>
<td>testosterone, inhibin</td>
</tr>
</tbody>
</table>
Receptors: How a target cell recognizes a hormone

Endocrine cell A

Hormone A

Hormone B

Receptor B

Target cell B

Not a target cell

Endocrine cell B

Receptor A

Target cell A
Hypothalamo-Pituitary Target Organ Systems
Endocrine Disruption

Effect of chemicals on endocrine function
Sites Where Endocrine Disruptors Can Act

- Hormone receptor
- Hormone secretion
- Hormone bioavailability, and clearance
- Feedback control
EDSP Tier 1 Battery of Screening Assays

**In vitro**

- ER binding (rat uterine cytosol)
- hERα transcriptional activation (ERTA) (HeLa-9903 cells)
- AR binding (rat prostate cytosol)
- Steroidogenesis H295R (human adrenocortical tumor)
- Aromatase (human recombinant microsomes)

**In vivo**

- Uterotrophic (rat, sc, ovex, uterine wt.)
- Hershberger (rat, oral, orchidex, peripubertal, sex organ wts)
- Pubertal female (rat, oral, time of vaginal opening, TSH, T4, etc.)
- Pubertal male (rat, oral, preputial separation, TSH, T4, etc.)
- Amphibian metamorphosis (tadpole to frog, thyroid histology)
- Fish short-term reproduction (male, female fathead minnows, morphological and biochemical endpoints)
Other Assays Relevant to Endocrine Disruptor Assessment

• Multigeneration studies

• In utero effects on offspring
Receptor Binding Does Not Predict The Nature of a Biological Effect
# Diversity of Biological Effects of ER Ligands

<table>
<thead>
<tr>
<th>ER ligand</th>
<th>Mammary gland</th>
<th>Uterus</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-estradiol</td>
<td>mitogenic</td>
<td>mitogenic</td>
<td>anti-osteoporotic</td>
</tr>
<tr>
<td>tamoxifen</td>
<td>anti-mitogenic</td>
<td>mitogenic</td>
<td>anti-osteoporotic</td>
</tr>
<tr>
<td>raloxifene</td>
<td>anti-mitogenic</td>
<td>anti-mitogenic</td>
<td>anti-osteoporotic</td>
</tr>
</tbody>
</table>

ER Mechanism of Action

- Cell membrane
  - Cytoplasm
    - Ligand
      - ER
      - Co-regulatory proteins
    - ER-ligand complex
      - Nucleus
        - Genome
      - Cellular response
Factors Contributing to Diversity of Response of Estrogen Receptor Ligands

• Heterogeneity of ER subtypes (ER\textsubscript{\alpha}, ER\textsubscript{\beta})
• Ligand specific alterations in ER conformation
• Heterogeneity of co-regulatory proteins
• Heterogeneity of ER binding sites within the genome (estrogen response elements)
• Alternate (non-genomic) pathways
• ER signaling may “cross-talk” with other pathways (e.g., aryl hydrocarbon)
Species Differences May Be Important
## Endocrinology of Pregnancy: Murine vs. Human

*(Witorsch, RJ, Food and Chemical Toxicology 40: 905-912, 2002)*

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Murine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominance of corpus luteum</td>
<td>Throughout gestation</td>
<td>First trimester <em>(luteal-placental shift)</em></td>
</tr>
<tr>
<td>Adrenal DHEA as estrogen precursor</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Estrogens produced</td>
<td>Estradiol, estrone</td>
<td>Estradiol, estrone, <em>estriol</em></td>
</tr>
<tr>
<td>Relative bioavailable estrogen</td>
<td>1</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>
Human vs. Murine Gestation

• Basic physiological differences
  – Role of corpus luteum
  – Pathways of estrogen production
  – Types of estrogens produced
  – Levels of bioavailable estrogen ($<100$-fold higher in humans)

• In utero species differences suggest that humans might be resistant to adverse effects of environmental estrogens observed in murines.
Stress, A Potential Confounder in Endocrine Disruptor Assessment

**Secretory Patterns of ACTH and Glucocorticoid**

**Basal** (diurnal or circadian): Recurring increase followed by a decrease every 24 hrs as a function of subject’s activity pattern.

**Stress-induced**: Abrupt or prolonged increase in response to homeostatic disruption (or “stressor”).
Effects of Stress or Glucocorticoid Excess on Reproduction and Development

- Testicular dysfunction
  - impaired testosterone production and Leydig cell apoptosis
- Ovarian hypofunction, amenorrhea, and infertility in women and comparable effects in animals
  - a “multistage” effect on the hypothalamo-pituitary-ovarian axis.
- Fetotoxic effects and post-partum “programming”

*Cooke, Holsberger, Witorsch et al., Toxicology and Applied Pharmacology 194: 309-335, 2004*
Effects of Stress or GC Excess In Utero on Offspring

- Intrauterine growth retardation (IUGR)
- Suppression of testosterone “surge” from fetal testes
- Postnatal endocrine/reproductive and other effects (programming)
  - Insulin resistance and hypertension
  - Feminization of sexually dimorphic areas of hypothalamus of male offspring
  - Behavioral changes (decreased copulatory behavior of adult males)
  - Decreased anogenital distance, delayed or abnormal testicular descent and decreased testicular weight in male offspring
How Stress Can Confound Endocrine Disruptor Assessment

- Effects attributable to endocrine disruptive chemicals (EDCs) might be due to direct effects of stress-induced elevation of glucocorticoids.
- Putative EDC might evoke a nonspecific stress response activating the hypothalamo-pituitary-adrenocortical (HPA) axis.
- Putative EDC might activate element of HPA axis.
- Procedures performed during an assay (e.g. oral gavage, restraint) might activate HPA axis.
- Activation of HPA can produce false positive or false negative effects.
Summary and Conclusions

• A brief overview of the essentials of endocrine physiology was presented.
• Endocrine disruption was discussed with regard to the following considerations:
  – Diversity of biological effects produced by ER ligands;
  – Species differences (murine vs. human gestation);
  – The influence of stress (glucocorticoids) on reproductive function and development of offspring.
• The above are important considerations in screening for endocrine disruptors.
Thank You