DIET, AGING, and MIND
Part II

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Outline: Diet, Aging, and Mind

1. Review
2. Brain Changes in Aging
3. Dementias
4. Dietary Interventions: DHEA and Soy
5. DHEA
6. Soy
7. Summary and Conclusions
DHEA: Mechanism of Action

➢ Non-Genomic: Cell Surface

- GABA-A Receptor
  - CI⁻ Channel
  - Penatameric Structure: α, β, γ, δ, ε, ρ
  - Regional heterogeneity in Structure
  - Multiple Binding Sites
  - Direct and Indirect Effects

➢ Genomic: Transcription

- Androgen Receptor
  - Intracellular Trafficking
  - Transcriptional Activity
  - Ligand Dependent
Functional Assays

- Inter-female Aggression
- Androgen Receptor: Immunochemistry and Western Blot
- Gene Expression: CAT Reporter
- AR Intracellular Trafficking: Confocal Microscopy
- DNA Microarray: Gene Regulation
- PCR: Microarray Validation
1. In Vivo Studies in Female Mouse
Females were ovariectomized, given DHEA or DHT for 19 days, and tested for aggression toward OVX females. Three hours after the test, the animals were sacrificed and brains were harvested for limbic system AR and ERβ determinations via Western blot.
DHEA and DHT Upregulate AR in Female Mouse Brain

Ovariectomized female CF-1 mice were implanted with pellets containing DHEA (472 ug/day), DHT (72 ug/day), or placebo for 15 days. ICC for AR was conducted using PG-21 antibody according to methods in Lu et al. (1999).
CF-1 female mice were ovariectomized and treated S.C. with DHEA (0.5 mg daily release), T (0.1 mg daily release), or placebo pellet for seven days. AR immunoreactive cells are distributed only in the boundary of CA1 pyramidal cell layer.
2. GENE REPORTER ASSAYS
DHEA Metabolism

- **DHEA Sulfate**
  - Sulfotransferase
  - Sulfatase

- **Dehydroepiandrosterone**
  - 3βHSD
  - 7α-hydroxylase
  - 7α-OH-DHEA

- **Androstenedione**
  - 17βHSD
  - P450 aromatase
  - Estrone

- **Testosterone**
  - 17βHSD
  - P450 aromatase
  - Estradiol
DHEA and Major Metabolites: CAT Activity via AR

AR-mediated CAT activity induced by DHEA and its metabolites

Relative CAT Activity (Fold)

Veh  7α-OH-DHEA  7β-OH-DHEA  7-oxo-DHEA  DHEA-S  DHEA

Treatment Concentration: $-\log_{10} (M)$

Involved plasmids: pSG5-AR, pMMTV-ARE-CAT

Mo et al., 2006
Reporter Assays: Summary

- DHEA is androgenic
  - Conferred AR transcriptional activity in a dose-dependent manner, and the effect was inhibited by flutamide.

- DHEAS, 7α-, 7β-OH-DHEA and 7-oxo-DHEA are not androgenic
3. INTRACELLULAR TRAFFICKING
Intracellular Trafficking: Time Course

DHEA

Adiol

Adione

90 Minutes Post-treatment

DHEA 10-5  Adiol 10-6  Adione 10-6

Mo et al. (2006)
Nuclear Distribution of AR-GFP in COS-7 Cells Following Androgen or Androgen + Flutamide Treatment

Androgen

10^{-7} M DHEA

10^{-7} M Adiol

10^{-7} M Adione

Androgen + Flu

DHEA + FLU

Adiol + FLU

Adione + FLU
DHEA is androgenic

- Conferred AR transcriptional activity in a dose-dependent manner and the effect was inhibited by flutamide.
- Promoted intracellular trafficking of AR with inhibition by flutamide

DHEAS, 7α-, 7β-OH-DHEA and 7-oxo-DHEA are not androgenic
Microarray Analysis
Genomic Analysis of DHEA- and DHT-Regulated Gene Expression in the Mouse Hypothalamus and Hippocampus

**Experimental Design**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>DHEA</th>
<th>DHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 + 4 mice</td>
<td>4 + 4 mice</td>
<td>4 + 4 mice</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Control</th>
<th>DHEA</th>
<th>DHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>A1, A2</td>
<td>B1, B2</td>
<td>C1, C2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>D1, D2</td>
<td>E1, E2</td>
<td>F1, F2</td>
</tr>
</tbody>
</table>

* Four brain regions were pooled to make one RNA samples
Heirarchical Cluster Analysis of Differentially Expressed Genes in Hypothalamus and Hippocampus

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>DHEA/Veh Hypothalamus</th>
<th>DHT/Veh Hypothalamus</th>
<th>DHEA/Veh Hippocampus</th>
<th>DHT/Veh Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepro-MCH</td>
<td>3.50</td>
<td>2.02</td>
<td>1.30</td>
<td>1.50</td>
</tr>
<tr>
<td>Hypocretin</td>
<td>2.41</td>
<td>1.59</td>
<td>1.03</td>
<td>1.01</td>
</tr>
<tr>
<td>PKCδ</td>
<td>1.43</td>
<td>1.81</td>
<td>1.01</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Real-time RT-PCR Analysis of Gene Expression in Mouse Hypothalamus

(a) Prepro-MCH at Hypo

(b) Orexin at Hypo

(c) PKCd at Hypo

(d) B2MT at Hypo
Summary

- DHEA is androgenic
  - Conferred AR transcriptional activity in a dose-dependent manner and the effect was inhibited by flutamide.
  - Promoted intracellular trafficking of AR with inhibition by flutamide

- DHEAS, 7α-, 7β-OH-DHEA and 7-oxo-DHEA are not androgenic

- DHEA alters the expression of major genes involved in energy utilization, alertness, appetite and cell death including orexin, prepro-MCH, and PKC-delta
Overall Summary

- DHEA inhibits female-typical, non-androgen dependent aggression
- Behavioral effects of DHEA and DHT differ, suggesting differences in mechanism of action
- DHEA is androgenic *in vivo* and *in vitro*
- The DHEA-AR complex acts as a transcription factor
- A cross-talk signaling pathway exists for DHEA in the CNS
Soy, Physiology, and Behavior
Diseases Caused by Atherosclerosis are the Leading Cause of Illness and Death in the United States

- Coronary artery disease (heart attack and angina)
- Stroke or transient ischemic attack
- Peripheral arterial disease
- Renovascular hypertension
Atherosclerosis
Soy Phytoestrogens: ERβ Mediation

• Isoflavones preferentially bind ERβ

• ERβ is a modulator of ERα in several systems

• Isoflavones may decrease 5-HT activity in dorsal raphe
## Table 1 Phenotypes of estrogen receptor knockout mouse (ERKO) models

<table>
<thead>
<tr>
<th>Tissue</th>
<th>αERKO</th>
<th>βERKO</th>
<th>αβERKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both sexes are infertile</td>
<td>Males are fertile</td>
<td>Both sexes are infertile</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>Hypoplastic uterus; insensitive to estradiol; no implantation</td>
<td>Normal response to estradiol; supports normal pregnancy</td>
<td>Resembles αERKO phenotype; insensitive to estradiol</td>
</tr>
<tr>
<td>Ovary</td>
<td>No ovulation; immature follicles; hemorrhagic cysts developing at puberty due to chronic elevated LH; elevated levels of estrogen and testosterone</td>
<td>Normal appearance; reduced ovulation</td>
<td>Granulosa cells undergo transdifferentiation into Sertoli-like cells</td>
</tr>
<tr>
<td>Mammary Gland</td>
<td>Immature; only a ductal rudiment present</td>
<td>Normal structure; normal lactation</td>
<td>Immature; resembles αERKO phenotype</td>
</tr>
<tr>
<td>Testes</td>
<td>Normal development; testes weight decreases with age; fluid retention and dilation of seminiferous tubules; sperm have poor motility</td>
<td>Normal</td>
<td>Resembles αERKO phenotype</td>
</tr>
<tr>
<td>Mating Behavior</td>
<td>Decreased aggression; disrupted mating behavior</td>
<td>Normal sexual behavior</td>
<td>Males do not mount; disrupted mating behavior</td>
</tr>
<tr>
<td>Bone</td>
<td>Both sexes are shorter than wild-type; females have decreased bone diameter; males have decreased density</td>
<td>Females have increased density; normal in males</td>
<td>Both sexes are shorter than wild-type</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Estrogen protection retained in vascular injury study</td>
<td>Estrogen protection retained in vascular injury study</td>
<td>Estrogen protection lost in vascular injury study</td>
</tr>
</tbody>
</table>

*αLH, luteinizing hormone.*
Reproductive Tissues of the Adult Female Estrogen Receptor Knockout Models

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>αERKO</th>
<th>βERKO</th>
<th>αβERKO</th>
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</thead>
<tbody>
<tr>
<td><strong>Uterus</strong></td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
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</tr>
<tr>
<td><strong>Ovary</strong></td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td><strong>Mammary Gland</strong></td>
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</tbody>
</table>

ERKO, estrogen receptor knockout mouse. Walker and Korach ILAR 45
Dietary Soy Phytoestrogens and Agonistic Behavior in Male Cynomolgus Macaques

• N = 44 male cynomolgus macaques
• Low, high, or no soy isoflavone diet for 15 months
• Pretreatment observation for 8 weeks: aggressive, submissive, and affiliative behaviors
• Retest at end of diet: behavior and hormonal response to GnRH
Severe Agonistic Behavior in Cynomolgus Males fed Control or Soy Protein Diets containing 0.94 (low) or 1.88 mg/g (high) isoflavone for 15 months

* p < 0.05 relative to C/L group. Simon, N. et al. 2004
Why is this Important?
Replacement Therapy: Male

That Sinking Feeling
Hair isn’t the only thing that falls with age; the hormones plentiful in youth also drop.

PERCENTAGE OF PRODUCTION IN MEN

![Graph showing percentage of production in men for Testosterone and DHEAS*]

Soon after I inject myself with testosterone I feel a deep surge of energy. My attention span shortens. My wit is quicker, my mind faster, but my judgment is more impulsive.
Replacement Therapies: Female

Wyeth Bros Elixir

- PREMARIN® (conjugated estrogens tablets, USP) 0.625 mg
- PREMPRO™ (conjugated estrogens/dydroxyprogesterone acetate tablets) 0.625 mg / 2.5 mg
- PREMPHASE™ (conjugated estrogens/dydroxyprogesterone acetate tablets) 0.625 mg / 5 mg
- PREMARIN® (conjugated estrogens) VAGINAL CREAM in a nonliquefying base
Health and Social Issues

The Aging Mind
Alzheimer’s Disease & Normal Aging