COMPUTATIONAL MODEL OF STEROIDOGENIC PATHWAYS IN FISH GONADS

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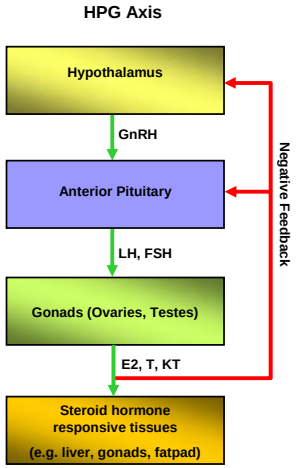
ABSTRACT

Exposure to endocrine disrupting compounds (EDC) can induce adverse effects on reproduction mediated through alterations in the enzymes involved in steroidogenesis. We are developing a computational model of the intratesticular and intraovarian metabolic network that mediates steroidogenesis to identify and link new molecular biomarkers of exposure to effects for EDCs. The mathematical model describes the biosynthetic pathways for the conversion of cholesterol to the primary steroid hormones (testosterone, estradiol, and 11-ketotestosterone) secreted by the testes and ovaries in fish. The model includes the intermediate molecules and reactions for the multiple pathways involved in the biosynthesis of the primary steroid hormones. The initial concentrations and enzyme kinetic reaction rates are taken from the literature or set to biologically reasonable values. This model allows for an improved understanding of the source-to-outcome linkages necessary for effective use of molecular biomarkers for risk assessments with EDCs. Since the biosynthetic pathways for steroid hormones are to a significant extent evolutionarily conserved, this computational model is likely to also be relevant for mammalian species.

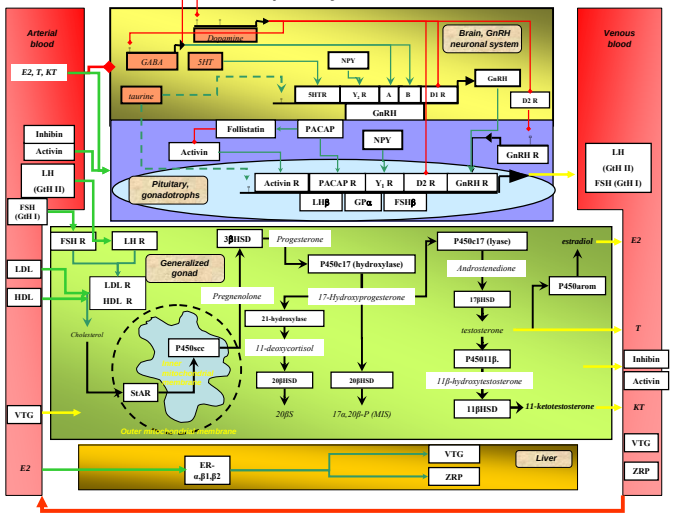
LINKING BIOMARKERS OF EXPOSURE TO EFFECTS

	Molecular	Cellular	Organ	Individual	Population
Biological Effects	Receptor-ligand interaction, DNA binding, enzyme activity	Altered signaling, gene activation, protein synthesis	Altered physiology and tissue morphology	Impaired development and reproduction, cancer, death	Structure, Extinction
Biomarkers	mRNA, protein, enzyme levels	Metabolite profiles	Functional and structural change (pathology)	Altered reproduction or development	Decreased number of animals
Computational model	Systems biology models				
Small fish model	Fathead Minnow Partially characterized genome High ecological/regulatory relevance Molecular markers, metabolomics				

HYPOTHALAMIC-PITUITARY-GONADAL (HPG) AXIS



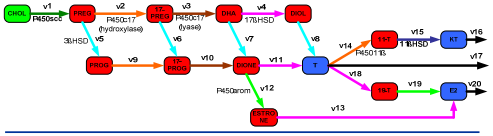
Feedback control system of the HPG axis that regulates synthesis and secretion of primary steroid hormones (estradiol (E2), testosterone (T), and 11-ketotestosterone (KT, only in male fish)) by the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, and luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary.



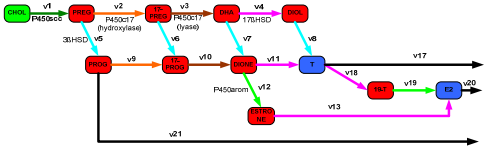
Conceptual systems model shows key regulatory components of HPG axis. Green and red arrows indicate activation and negative feedback (inhibition), respectively. White boxes indicate proteins and peptides. Small molecules (e.g. steroids and neurotransmitters) are shown in italics.

COMPUTATIONAL MODEL

Intratesticular Steroidogenic Pathway



Intraovarian Steroidogenic Pathway



- Predominantly one-way reactions
- Secretion rates of intermediates are negligible

Deterministic Model

$$\frac{d}{dt}CHOL = -v1 \qquad \frac{d}{dt}PROG = v9 - v5 \qquad \frac{d}{dt}19T = v18 - v19$$

$$\frac{d}{dt}PREG = v1 - v2 - v5 \qquad \frac{d}{dt}17PROG = v6 + v9 - v10 \qquad \frac{d}{dt}E2 = v13 + v19 - v20$$

$$\frac{d}{dt}17PREG = v2 - v3 - v6 \qquad \frac{d}{dt}DIONE = v10 + v7 - v11 - v12 \qquad \frac{d}{dt}11T = v14 - v15$$

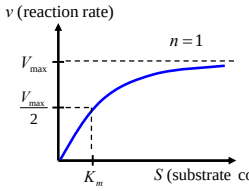
$$\frac{d}{dt}DHA = v3 - v4 - v7 \qquad \frac{d}{dt}ESTRONE = v12 - v13 \qquad \frac{d}{dt}KT = v15 - v16$$

$$\frac{d}{dt}DIOL = v4 - v8 \qquad \frac{d}{dt}T = v11 + v8 - v14 - v17 - v18$$

Enzyme Kinetics

$$v_i = \frac{V_{max,i}\, S_i^n}{S_i^n + K_{m,i}}$$

2 parameters: V_{max}, K_m



Model Parameters

(8 unique reaction rates x 2 parameters per reaction) + 3 secretion rates = 19 total parameters

EDC EXPOSURES

Small fish exposure system

Fathead minnows

- Exposure of male and female fathead minnows to EE2 (synthetic estrogen): high ecological/regulatory relevance
- Dose levels: 0 (control), 10, 100 mg/L
- Dosing phase: 8 days
- Recovery phase: 8 days
- Tissue sampling: day 1, 4, 8, and 16

PARAMETER ESTIMATION

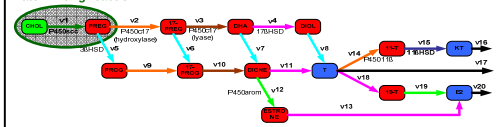
Objective function: $f = \sum_{i=1}^{I}\sum_{n=1}^{N}\left[S_{i,n} - S_i(t_n, \theta)\right]^2$

where: I = number of species (metabolites)
N = number of time samples
S = concentration of species (metabolite)
θ = adjustable model parameters

- Apply an iterative optimization algorithm
- Estimate parameters separately for male and female models
- Simultaneously estimate parameters from all dose levels

MODEL SIMPLIFICATION

Rate-limiting reaction



- Motivation
  - More intuitive understanding of dynamic functional behavior
  - Reduces number of model parameters
- Method
  - Identify rate limiting step(s): quasi-steady state approximations
  - Identify preferred pathways

DISCLAIMER

This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect Agency policy.

Building a scientific foundation for sound environmental decisions