Computational Model of Adrenal Steroidogenesis to Predict Biochemical Response to Endocrine Disruptors

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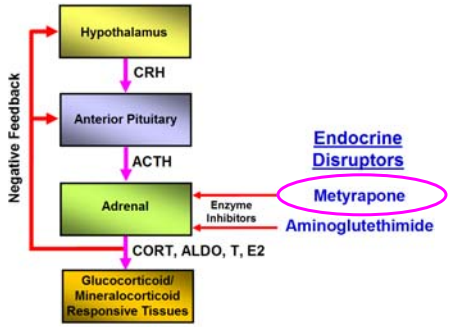
ABSTRACT

Steroids, which have an important role in a wide range of physiological processes, are synthesized primarily in the gonads and adrenal glands through a series of enzyme-mediated reactions. The activity of steroidogenic enzymes can be altered by various endocrine disruptors (ED), some of which are environmental contaminants. We developed a dynamic computational model of the metabolic network of adrenal steroidogenesis to predict the synthesis and secretion of adrenocortical steroids, and the biochemical responses to ED. The deterministic model describes the biosynthetic pathways for the conversion of cholesterol to adrenocortical steroids, and the kinetics for enzyme inhibition by the ED, metyrapone. Experiments were performed using H295R human adrenocarcinoma cells to measure concentrations of 14 steroids using LC/MS/MS and ELISA methods, and model parameters were estimated using an iterative optimization algorithm. Model-predicted steroid concentrations closely correspond to the dynamic dose-response data from the experiments. A sensitivity analysis of the model parameters identified metabolic processes that most influence the concentrations of the primary steroid produced by the adrenal gland: cortisol. Our study demonstrates the feasibility of using the computational model of adrenal steroidogenesis to predict the *in vitro* adrenocortical steroid concentrations using H295R cells. This capability could be useful to help define mechanisms of action for poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system, and predictive risk assessments.

EFFECTS OF ENDOCRINE DISRUPTORS ON HPA AXIS



Feedback control system of hypothalamus-pituitary-adrenal (HPA) axis regulates synthesis and secretion of adrenocortical steroids (cortisol (CORT), aldosterone (ALDO), testosterone (T), estradiol (E2)) by release of corticotropin releasing hormone (CRH) from hypothalamus, and adrenocorticotropic hormone (ACTH) from pituitary

H295R CELL LINE

- Established from human female adrenocortical carcinoma
- Maintains ability to secrete all major adrenocortical steroids
- Proposed as EPA screening assay for environmental chemicals capable of disrupting or modulating steroidogenesis
- Being evaluated to screen drug candidates based on steroidogenic effects in early phase of drug development

CELL PROLIFERATION EXPERIMENTS

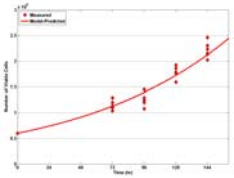
- Incubate cells in medium for 72 hr
- Incubate cells in new medium + stimuli + carrier for 72 hr
- Collect samples at 0, 72, 96, 120, 144 hr
- Measure number of viable cells using cell viability analyzer

CELL PROLIFERATION MODEL

$$\frac{dN}{dt} = k_p N \Rightarrow N = N_0 e^{k_p t}$$

N = number of viable cells
k_p = growth rate
N_0 = initial number of viable cells

Estimated: $k_p = 0.00878$



- Direct least-squares solution by linearization

STEROIDOGENESIS EXPERIMENTS

- Incubate cells in medium for 72 hr
- Start clock: Incubate cells in new medium + stimuli + carrier + ED for 72 hr

Metyrapone Study

- Baseline and two metyrapone concentrations (1, 10 μM)
- Collect samples at 0, 8, 24, 48, 72 hr
- Measure cell and medium concentrations of E1 and E2 using ELISA and remaining 12 steroids using LC/MS/MS

STEROIDOGENESIS MODEL

- Mathematical model based on *in vitro* experimental design
- Two compartments: medium and H295R cells
- Two pathways: transport and metabolism
- First-order metabolic reaction rates
- Assumed quasi-equilibrium for steroid transport
  - rapid & reversible steroid transport between medium and cells
- First-order cholesterol transport rate
- Endocrine disruptors: partition coefficient for transport, competitive enzyme inhibition

Equilibrium Equations

$$C_{x,\text{med}} = q_x C_{x,\text{cell}}$$

q_x = equilibrium constant for steroid x
$C_{x,\text{cell}}$ = concentration of steroid x in cells
$C_{x,\text{med}}$ = concentration of steroid x in medium

- Yields algebraic equations
- Decouples equations for steroids in medium from equations for steroids in cells
  - simplify large inverse problem

Dynamic Molecular Balances

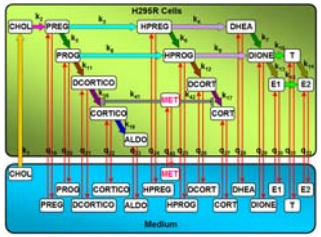
$$\frac{d}{dt}\left(V_{\text{cell}} C_{x,\text{cell}} + V_{\text{med}} C_{x,\text{med}}\right) = P_{x,\text{cell}} - U_{x,\text{cell}}$$

V_{cell} = volume of viable cells
V_{med} = volume of medium
$P_{x,\text{cell}}$ = production rate of steroid x in cells
$U_{x,\text{cell}}$ = utilization rate of steroid x in cells

Competitive Enzyme Inhibition Equation

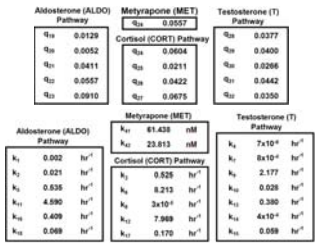
$$v_i = \frac{k_i}{1 + \frac{C_{\text{ED,cell}}}{k_{\text{ED},i}}}$$

v_i = overall metabolic reaction rate for reaction i
k_i = first-order metabolic reaction rate for reaction i
$C_{\text{ED,cell}}$ = concentration of ED in cells
$k_{\text{ED},i}$ = enzyme inhibition constant of ED for reaction i

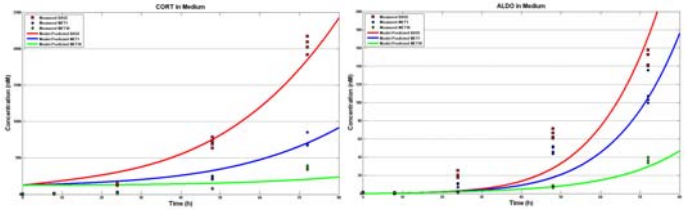


- 17 first-order metabolic reaction rates
- 14 steroid equilibrium constants
- First-order CHOL uptake rate
- MET partition coefficient
- 2 enzyme inhibition constants

MODEL PARAMETERS

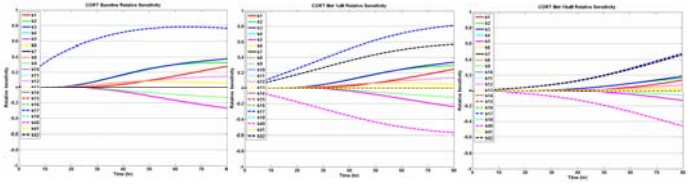


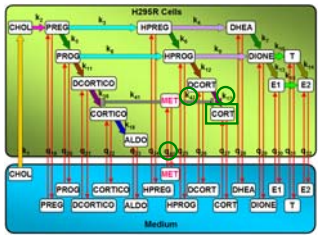
ASSESSMENT OF MODEL FIT



Comparison of model-predictions with time-course data from baseline and two metyrapone concentrations (1, 10 μM). Model-predicted concentrations of cortisol and aldosterone in the medium were plotted as a function of time, and compared with concentrations measured at five time points.

SENSITIVITY ANALYSIS





Graphical representation of parameters with highest sensitivities (green circles) for cortisol.

Relative sensitivities for model outputs of cortisol in medium are plotted as function of time for 21 parameters for baseline and two metyrapone concentrations (1, 10 μM). Negative values indicate an inverse relationship between a parameter change and resulting model output change; positive values indicate a direct relationship. Magnitudes indicate degree to which changes in parameter values lead to change in model outputs; percentage change of model output for a given percentage change of parameter.

DISCUSSION

- Steroidogenesis model can predict dynamic steroid concentrations in H295R cells for baseline and two metyrapone doses
- Mechanistic model can improve understanding of dose-response behavior for environmental chemicals that alter activity of steroidogenic enzymes
- This capability could help define mechanisms of action for poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system