Development of a Biologically Based Pharmacokinetic (BBPK) Model for the Hypothalamic-Pituitary Thyroid Axis in the Maturing Rat for the Dose Response Assessment of Developmental Neurotoxicity

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Multifaceted Objectives of Research

Modeling (Jeff Fisher, Eva McLanahan, Libby Myers)
1) To better understand relationships between administered dose and HPT axis disturbances in the immature rat and neurodevelopmental toxicity:
   A. Biologically based models of the HPT axis are under development for different reproductive states of rats, adult male rats, and human.
   B. PBPK models for thyroid active chemicals will be linked to the HPT axis models to predict neuro-developmental toxicity endpoints.

Experimental (Duncan Ferguson, John Wagner, Matthew Taylor, Michael Stramiello, Nadia Paolino)
2) Using gestational/neonatal exposure of rats to thyroid disruptive compounds, to:
   A. Examine the sensitivity, capacity and development of compensatory mechanisms of thyroid hormone secretion/metabolism by the thyroid, brain, and liver.
   B. Develop quantitative ‘dose-response’ relationships of serum and tissue markers of thyroid status and correlate with developmental neurotoxicity endpoints.
Approach - Cooperative agreements

- Develop team of interdisciplinary scientists; while working independently, are aiding each other in experimental design, sharing samples and data.

-- UGA, UMass (Tom Zoeller) and USEPA (Kevin Crofton, Mike DeVito, and Mary Gilbert)
**Project Concept for Computational Modeling of Dose-Response in the Fetal/Neonatal Rat**

- **PBPK**
  - Describes the kinetics of the toxicant and its MOA for disturbing the HPT axis.

- **BBPK**
  - Thyroid axis
  - Describes the HPT axis and perturbations in the HPT axis from chemical insult.

- Dose of Toxicant

- CNS responses in the brain of the pup or fetus.

- % CNS Toxicity vs. Internal Dosimetrics
  - Dose-Response

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Approach for Computational Modeling

...Fill fundamental D-R data gap (HPT disruption \(\Rightarrow\) hypothyroidism \(\Rightarrow\) developmental neurotoxicity)

- Use propylthiouracil (PTU), as a probe to establish high to low dose quantitative relationships between disruption of the HPT axis leading to hypothyroidism in the developing rat and neurotoxicity.

...Select two thyroid active chemicals with substantial data

- Perchlorate (iodide blocking at thyroid) and PCB 126 (increased hepatic T4 metabolism). Both environmental chemicals cause hypothyroidism in rats.
Sub models

_______BBPK- HPT axis_________
Linking the Sub Models - I see the light

• PBPK models
  • PBPK dosimetry model with MOA (perchlorate and PCB 126)
    -- adopt aspects of perchlorate PBPK models

• Pup growth PBPK model using growth equations for organs
  -- adopt in-house research from deltamethrin
  • Utilize in-house PCB 126 kinetic data sets

HPT axis models
  • Recalibrate radiolabeled iodide submodel, calibrate radiolabeled T4 and T3, calibrate endogenous TSH submodel

• Develop endogenous $^{127}$I, T3, T4 and link with TSH (feedback)

• Articulate compensatory mechanisms for HPT axis such as T3/T4 shift in thyroid hormone production, D2 induction in brain, NIS induction in thyroid, extra-thyroidal D1 decrease
Current Status of Models (two Ph.D. students)

- Develop radiolabel sub-models for iodide and T4 in PND 13 pup and adult rat.
- Examine feedback equations for endogenous serum TSH and T4 concentrations.
- Dietary iodide model for adult human
${^{125}}I$ Model Structure
Approach for Iodide Binding in Thyroid

![Diagram of thyroid with equations and graphs]

\[
\frac{dIB}{dt} = \frac{V \max_b t_i * C_{f_i}}{K_{m_b} - i + C_{f_i}}
\]

Binding equation

\[
V \max_b t_i = \frac{V_{0 bind} * K_{aib}}{K_{aib} + C_{IB_i}}
\]

Binding inhibition equation

\[
\frac{dIB}{dt} = \frac{dIB}{dt} - \frac{dTHprod}{dt}
\]

Rate of change in amount bound

Adapted from Pedraza et al., 2006 and Nagataki et al., 1966
Bound $^{125}$I in Thyroid

[Graph showing the time course of bound $^{125}$I in the thyroid of a PND 14 pup following a 1 ug/kg oral gavage dose, and an adult male rat following a 33ug/kg i.v. dose.]

Clewell et al., 2003

Yu et al., 2002
$^{125}$I PBPK Model Predictions

PND 14 Pup
1 ug/kg oral gavage dose

Adult Male Rat
33 ug/kg i.v. dose

Clewell et al., 2003

Yu et al., 2002
[125I]-T4 PBPK Model Structures and Predictions

Adult Male Rat
4.4 ng i.v. dose
Wong et al., 2005

PND 14 Pup
9.6 ng i.v. dose
Silva et al., 1984
Thyroid (negative feedback loop)

\[ V_d \left( \frac{dT_4}{dt} \right) = \left( k_0 + \frac{k_{0,\text{max}} \cdot [TSH]}{[TSH] + k_{TSH}} \right) - \left( k_{\text{cat}} \cdot [\text{UDP}GT] \cdot [T4] \right) - \left( k_{\text{cat}}' \cdot [\text{Deiod}] \cdot [T4] \right) \]

\[ V_{pl} \left( \frac{dTSH}{dt} \right) = \left( \frac{k_{0,TSH} \cdot k_{T4}}{k_{T4} + [T4]} \right) - \left( k_{\text{elim,TSH}} \cdot [TSH] \right) \]

Negative feedback, ↓T4, ↑TSH

Where

- \( k_0 \) = basal production rate of T4 (in absence of TSH)
- \( k_{0,\text{max}} \) = maximal rate of T4 secretion to plasma (under TSH stimulation)
- \( k_{0,TSH} \) = rate of TSH production (T4 conc. approaches 0)

↑TSH, ↑T4 production
Steady state prediction vs observation for serum T4 and TSH concentrations in adult male rat
Experimental Work
Duncan Ferguson
Blood / CSF Free T4

Nucleus

TRE Activation

Pre-translational regulation

Ribosomes

Increased in hypothyroidism

Nuclear Occupancy

OATP1C1

T3

Post-translational regulation

D2

T4

Glial Proteins

GFAP

MBP

Glial Cell Function

Myelogenesis

Astrocyte/Tanycyte

MCT8

T3

T2

Neuronal Proteins

RC3 Neurogranin, Synaptophysin

Neuronal Function, Growth, Differentiation, Synaptogenesis

LTP, Synaptic Response, Learning, Memory

Nucleus

TRE Activation

Ribosomes

Astrocyte/Tanycyte

Neuron
Dosing Protocol

- Dosing of timed pregnant Sprague-Dawley dams started at GD2 and continued through PND21-PND30 depending upon sacrifice schedule.
- Current dose levels are 0, 3, and 10 mg/L (ppm) PTU in the drinking water.
- Water intake recorded and animals weighed q48h.
- Gender of offspring determined in the third week after birth, and female pups culled midway through that week.
Timeline

- Pups were sacrificed from PND21-PND31.
- Dams were sacrificed on PND31, when the pups were weaned.
- Adults were sacrificed starting 2 months after weaning (average PND100)
- Female pups were culled on approximately PND 24; 2 males per litter studied at each timepoint
- 14 litters
  - 0 ppm (n= 5)
  - 3 ppm (n=5)
  - 10 ppm (n=4)
- Additional analyses for D2 activity were performed on Hooded Long-Evans rats (1 dam and 1 PND21 pup) dosed at 0 (n=12), 1 (n=13), 2 (n=13) and 3 (n=12) ppm PTU from GD6 to PND21.
Serum Thyroid Hormone and TSH Concentrations: Dams

- **Dam Serum T4**
  - Graph showing the effect of PTU dose (0.0, 3.0, 10.0 ppm) on T4 levels. Asterisks indicate statistical significance.

- **Dam Free T4 by Dialysis**
  - Graph showing the effect of PTU dose (0.0, 3.0, 10.0 ppm) on free T4 levels. Asterisks indicate statistical significance.

- **Dam Serum Total T3**
  - Graph showing the effect of PTU dose (0.0, 3.0, 10.0 ppm) on total T3 levels. Asterisks indicate statistical significance.

- **Dam Serum TSH**
  - Graph showing the effect of PTU dose (0.0, 3.0, 10.0 ppm) on TSH levels. Asterisk indicates statistical significance.
Serum Thyroid Hormone Concentrations: PND25

PND25 Serum Total T4

PND25 Serum T3

PND25 Pooled Serum Free T4
Hepatic D1 Activity

**Postnatal Day 25 Dams**
Hepatic Type I 5'-Deiodinase

**Postnatal Day 25 Rat Pups**
Hepatic Type I 5'-Deiodinase
PND25 Cortical T3 and D2 Activity: PND25 vs. Dam
Serum T4 vs. Cortical T3

Max = 2564+/−550 pg/g
T4 at Half Max = 0.88+/−0.52
R = 0.69
Comparison of D2 response to Fall in Total T4: PND21-30 vs. Dams

Total T4 vs. Cortical D2 Activity: PND 21-30

- UGA
- EPA

\[ y = C \times \exp(-k \times x) \]
C = 1401 +/- 153 (SD)
k = 0.533 +/- 0.107; D2 doubles with 1.3 ug/dl fall
R = 0.88  p<0.0001

T4 vs. Cortical D2 Activity: Dams

- UGA
- EPA

\[ y = C \times \exp(-k \times x) \]
C = 171 +/- 24 (SD)
k = 0.531 +/- 0.108; D2 doubles with 1.3 ug/dl fall
R = 0.86  p<0.0001
Comparison of Thyroid mRNA response: PND21-30 vs. Dams

**PND25 Pups**
- NIS mRNA Expression
  - PTU (ppm): 0, 3, 10
  - NIS18S Ratio
  - Significant differences indicated by asterisk (*)

**PND25 Pups**
- Tg mRNA Expression
  - PTU (ppm): 0, 3, 10
  - Significant differences indicated by asterisk (*)

**Dams**
- NIS mRNA Expression
  - PTU (ppm): 0, 3, 10
  - NIS18S Ratio
  - Significant differences indicated by asterisk (*)

**Dams**
- Tg mRNA Expression
  - PTU (ppm): 0, 3, 10
  - NIS18S Ratio
PND100 Serum Hormones

PND100 Serum Total T4

PND100 Serum Free T4 by Dialysis

PND100 Serum T3
PND100 Cortical T3, and D1 and D2 Activities

**PND100 Cortical T3**

- PTU Dose (ppm): 0.0, 3.0, 10.0
- pg/g: (data represented with bar graphs)

**PND100 Cortical D2 Activity**

- PTU Dose (ppm): 0.0, 3.0, 10.0
- fmol/l: (data represented with bar graphs)

**PND100 Cortical D1 Activity**

- PTU Dose (ppm): 0.0, 3.0, 10.0
- fmol/l/mg/hr: (data represented with bar graphs)
Hippocampal Electrophysiology: Stimulus/Response Curves: PND21-30

![Graph showing fEPSP S/R curve with stimulus intensity on the x-axis and slope on the y-axis. The graph includes data points for control, 3ppm, and 10ppm.]
Longterm Potentiation: PND100

LTP timecourse

- **1 x 100Hz**
- **3 x 100Hz**

- Control
- 3 ppm

Baseline
Serum T4 vs. Synaptic Response: PND21-30

![Graph showing the relationship between Total T4 (µg/dl) and Synaptic Response. The equation of the line is y = -0.319x - 1.118. The slope is -0.319 ± 0.093, the y-intercept is -1.118 ± 0.190, R = 0.57, p = 0.002.](image)
Cortical T3 vs. Synaptic Response: PND21-30

![Graph showing the relationship between Cortical T3 and Synaptic Response. The graph includes a line of best fit with the equation: Slope = -0.0002849 ± 0.0001057, Y-intercept = -1.193 ± 0.2035, and R = -0.484 p = 0.012.]
Correlation of D2 vs. Maximum Synaptic Response at PND21-30

![Graph showing the correlation between Cortical D2 Activity (fmol I-/min/mg) and srimax. The slope is 0.00122 ± 0.00022, the intercept is -2.41 ± 0.18, and R = 0.75 with p < 0.0001.](image)
Conclusions: Thyroid Parameters

- Thyroid hormone depletion by gestational/neonatal PTU exposure is ameliorated within the cerebral cortex by D2 induction, whereas hepatic D1 activity is maximally inhibited by 10 ppm PTU.
- Cortical T3 concentrations in PND21-30 pups were maintained in the euthyroid range until a fall of about 75% of serum T4.
- A highly significant negative exponential relationship was observed between serum T4 concentration and D2 activity, with a doubling in D2 with every 1.3 ug/dl fall in T4 in both dams and pups. The relative D2 maximal response was ~8-fold higher in the pups.
- Both cortical D2 and thyroid NIS mRNA induction, likely tissue biomarkers of T4 deficiency/TSH elevation, demonstrate greater sensitivity of the offspring to thyroid hormone deficiency.
- All serum and tissue thyroid parameters returned to normal following 2 months of PTU withdrawal.
Conclusions: Electrophysiology

- Baseline synaptic transmission was significantly reduced in the CA1 region of hippocampal slices obtained from PND21-30 rats under the ongoing influence of PTU exposure.
- Slices obtained from littermates allowed to mature in the absence of PTU until PND90-100 did not exhibit any persisting change in baseline synaptic transmission, however a significant reduction in the magnitude of LTP was observed.
- The decreased ability of the synapses to undergo synaptic plasticity even after the animal has recovered to euthyroid status suggests that although some of the acute impact of hypothyroidism can be restored, the potential remains for significant persisting impairments on the processing of information through neuronal networks.
- D2 enzymatic activity is tightly and positively correlated with synaptic potential at PND25, and may serve as a useful biomarker of thyroid hormone sufficiency in the brain.
Ongoing and Future Work

- Tissue thyroid markers
  - Cortical D3 activity
  - Tissue T4 concentrations
  - In situ hybridization: D2, D3, RC3, GFAP, MCT8, OATP1C1
- Thyroid markers
  - Histomorphometry
  - NIS and Tg immunohistochemistry
- Anatomical
  - Brain histopathology
  - Immunohistochemistry for BDNF, synaptophysin
- Refined dose studies: 0.3, 1 and 3 ppm
- Behavioral studies: locomotor and cognitive function as adults