Adrenal Gland: Preclinical Toxicologic Pathology and Effects of Stress

Thomas Rosol, DVM, PhD, DACVP
Veterinary Biosciences
Ohio State University, Columbus, Ohio, USA

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Presentation Outline

- Adrenal development
- Adrenal gland
  - Steroidogenesis
- Toxic injury of the adrenal gland
- HPA axis and the stress response
- Adrenal medulla and tumors
  - pheochromocytoma

Adrenal Organogenesis

- Adrenal and gonad: Same primordia
- Adrenal responds to ACTH; Gonad to LH
- Adrenal rests form in ovary when ACTH is high
- High LH can stimulate adrenal proliferation after switch to Gata-4 from Gata-6

Adrenal Glands

- Most common endocrine organ with chemically-induced lesions
- Understanding structure and function is important for interpreting the mechanisms and significance of chemically-induced lesions

Rat Adrenal Cortex
Zona Glomerulosa (Arcuata) 20%
Zona Fasciculata 65%
Zone Reticularis 15%
Homeostatic Model of Adrenocortical Growth

Adrenal Organogenesis
- Wnt from capsule targets Dax1 in proliferative cells
  - Dax1 required for adrenal differentiation
  - Cortisol prevents differentiation of proliferative cells
- Sf1 (steroidogenic factor 1): Marker for adrenal cortex; required for adrenal formation; induces CYP450 expression
  - Induced by ACTH

Zona Glomerulosa (Multiformis)
- Histology
  - Variable between species; glomeruli or arch-like (arcuata) cell cords
- Function
  - Production of mineralocorticoids
    - Aldosterone
      - Preservation of Sodium (Na⁺) (Blood volume)
      - Excretion of Potassium (K⁺)
- Required for life

Zona Fasiculata
- Histology
  - Columns (fasicles) of cuboidal to columnar cells along vascular sinusoids
- Function
  - Production of glucocorticoids (not required for life)
    - Cortisol
    - Corticosterone
- Endocrine control
  - CRH (Hypothalamus)
  - ACTH (Adenohypophysis)

Zona Reticularis
- Histology
  - Anastamosing cell cords
- Function
  - Production of glucocorticoids and small amount of sex steroids (estrogen, androgens)
- Endocrine control
  - CRH (Hypothalamus)
  - ACTH (Adenohypophysis)

Subcellular Structure/Function Relationships
- Lipid droplets (cholesterol esters)
- Smooth endoplasmic reticulum
- Mitochondria
- Minimal storage of hormone
- Steroids are hydrophobic molecules diffuse out of cells
Steroidogenesis

- **ACTH** (cAMP second messenger)
  - 1SAR protein (steroidogenic acute regulator protein)
    - Moves cholesterol to inner mitochondrial membrane
  - 1Cyp11A1
- **Mitochondria**
  - Side chain cleavage (Cyp11A1)
  - Hydroxylation to Pregnenalene
- **SER**
  - Converted to 11-Deoxycorticosterone
- **Mitochondria**
  - Hydroxylated to Corticosterone or Cortisol

Adrenal Cortex: Steroid Hormones

- 17-carbon steroid nucleus
  - Cholesterol is precursor
- Cytochrome P450 enzymes
  - Mitochondria, SER, Shuttling
- Secretion
  - Circadian rhythm
  - Nocturnal animals (rats, mice, cats): High at night
  - Daytime animals (dogs, humans): High in morning
  - Decreased secretion with age
- Bound in serum (90%) to CBG (transcortin)
- Metabolized in liver (hydroxylated and conjugated)

Glucocorticoid Secretion

- Pulsatile, ~ once per hour
- Circadian pattern
  - Peak concentrations at peak activity
  - Rodents peak during first few hours of the dark cycle
  - Rodent trough levels for first 6 hours during the light cycle
  - Diurnal patterns are lost in pregnant or lactating rats
- Concentrations vary with sex, strain, and age
  - Increase in rats after 3-4 weeks of age
  - Female rats have high concentrations of corticosterone and CBG
  - Aged rats have higher ACTH and corticosterone concentrations due to decreased brain glucocorticoid receptors

Major Glucocorticoids

- **Cortisol**
  - Fish (teleosts)
  - Hamsters
  - Dogs (similar to humans)
  - Cats
  - Nonhuman primates
  - Humans
- **Corticosterone**
  - Amphibians
  - Reptiles
  - Birds
  - Rats
  - Mice
  - Rabbits (also have cortisol, increases during stress)
ACAT: acyl-CoA:cholesterol acyltransferase, nCEH: neutral cholesterol ester hydrolase
Cynomolgus Monkey

Cynomolgus Monkey: CYP17A IHC

Cynomolgus Monkey: CYP11B2 IHC

Factors Contributing to Chemical Injury of the Adrenal Cortex

- Rich vascular supply
- High lipid content (steroidogenesis)
- Bioactivation by cytochrome P450 enzyme systems to reactive toxic forms
- Limited mechanisms of detoxification

Mechanisms of Toxicity

- Impaired Steroidogenesis
  - Disturbance of cholesterol metabolism
  - Cytochrome P450 disruption
  - Increased cytoplasmic lipid
- Toxin activation by CYP450
  - Phospholipidosis (esp. zona fasciculata)
- Exogenous steroids
  - Atrophy (increases negative feedback)
  - Proliferation (direct or indirect effect)
  - Blockage of peripheral receptors by antagonists
- DNA damage (carcinogens, radiation)

Chemical Inhibition of Steroidogenesis

- A, aminoglutethimide
- M, mitotane
- K, ketoconazole
- E, etomidate
- T, trilostane
- Me, metyrapone
Species Variations

- Sensitivity varies according to species

- Variation in pathways of steroid metabolism

- Variation in xenobiotic metabolism
  - Dogs and humans are similar (e.g. o,p'-DDD)
  - Rats susceptible to DMBA

Chemical-Induced Injury of the Adrenal Cortex

- Selected Examples of Mechanisms:
  - Inhibition of neutral cholesterol ester hydrolase

Inhibition of Neutral Cholesterol Hydrolase

**Triaryl Phosphate**

- Cholesterol lipidosis

- Adrenal cortical and ovarian interstitial cells

CORTICAL LIPIDOSIS: 3 WEEKS

**Triaryl Phosphate**

Mechanism: Inhibition of neutral cholesterol ester hydrolase

Lesions: Cytoplasmic lipid droplets in zona glomerulosa, reticularis, and fasciculata
Chemical-Induced Injury of the Adrenal Cortex

- Selected Examples of Mechanisms:
  - Disruption of organellar membrane turnover

Disruption of Organellar Membrane Turnover

- Examples:
  - Cationic Amphiphilic Compounds
    (Chloroquine, Triparanol, Chlorophentermine)
  - Toxin activation of CYP P450 enzymes
- Lesion: Phospholipidosis of cortical cells
  (vacuolation, need EM)

Cortical Phospholipidosis

Adrenocortical Tumors

(Human Relevance)

- Humans
  - Adenomas: 5% of people over 50 years, incidental, nonfunctional
    - Carcinoma is rare (500/yr in USA)
- Dogs: Similar to humans
  - Functional tumors more common
- Gonadectomy
  - Goats, ferrets, hamsters, and mice

The Stress Response

- Physiological response to a nonpredictable environmental change
- Activation of the HPA axis with an increase in glucocorticoids
- Maximizes survivability and suppresses nonessential functions
- Increased gluconeogenesis; decreased insulin sensitivity; diabetogenic
- Suppression of reproduction
- Regulation of immune function
- Increases activity and appetite

Interpreting Stress Responses during Routine Toxicity Studies: A Review of the Biology, Impact, and Assessment

- [Source](https://example.com)
Stress: Adrenal Hypertrophy

- Stress: Increases ACTH
  - Increased glucocorticoid synthesis and secretion
  - Increased utilization of neutral lipid (fat stores)
  - Cellular hypertrophy of the ZF and ZR
  - Increased proliferation and decreased apoptosis

Rat Adrenal Glands

Normal Hypertrophy

Stress and Preclinical Toxicology

- Stress responses in morphology and function
  - Confound interpretation of toxicology studies
- Adrenal glucocorticoids
  - Can influence the effects of toxic compounds
  - Exacerbate toxicity in the brain (Proinflammatory)
  - Reduce or protect against toxicity in other organs
    - Liver; Kidney; Heart
    - Anti-inflammatory
    - Membrane protective
    - Effects on blood glucose
    - Induction of antiapoptotic, antioxidant, and detoxification genes

Chemicals as stressors

- The HPA response is not stressor-specific
- Chemicals can alter homeostatic balance
  - Organ effects
  - Metabolism effects
- High doses of test articles
- Examples in wildlife from environmental contamination
  - Petroleum hydrocarbons; Organochlorines; Organophosphates; Metals
Increased Adrenal Gland Weight (Adrenal Hypertrophy)

- Stress
  - Known exposure to stress-inducing stimuli
  - Histopathology (Morphology)
  - Concurrence with other stress-related changes (e.g., thymic atrophy)
  - Clinical chemistry
    - Functional challenge

- Differential Diagnosis: Toxic Effect
  - Histopathology (Morphology)
  - Clinical chemistry
    - Functional challenge (functional suppression)
  - Mechanistic studies of adrenal cell function (steroidogenic enzymes)

Adrenal Hypertrophy: Significance

- Stress-Related
  - Primary and secondary stress-related effects
  - Less significant finding in toxicology study
  - Cause should be identified
  - Functional significance could be tested

- Test Compound-Related
  - Potentially serious
  - Understand mode of action
  - Human relevance
  - Species differences

Assessment of the HPA Axis

- ACTH challenge assay
  - Tests the integrity of the HPA axis through steroid hormone secretion

- Evaluation of in vitro mechanisms
  - H295R human adrenal cortical cells
    - Enzyme inhibition
      - CYP17, not well expressed in rodent adrenal glands
    - StAR function (steroidogenic acute regulatory protein; transport protein for cholesterol in mitochondria and rate limiting step in steroidogenesis)
    - Species differences in susceptibility to toxicants

ADRENAL MEDULLA

- Less common site of toxic manifestations
- Proliferative lesions
  - Important in the rat
  - Less common in the mouse
- Intermingling of cortical and medullary tissue
  - Human, pig, some strains of rats

Epinephrine  Norepinephrine

**Epinephrine (Catecholamine)**

**Norepinephrine (Catecholamine)**

\[
\text{Cortisol} \rightarrow \text{Medulla} \rightarrow \text{Epinephrine} \quad \text{PNMT}^* \rightarrow \text{Norepinephrine}
\]

*Phenylethanolamine N-Methyl Transferase*
ADRENAL MEDULLA: NEOPLASMS

• Secretory Cells
  – Pheochromocytoma (benign)
    • May start as hyperplasia
    • Expanding medullary mass
    • Well differentiated
    • Behavior
      – Space-occupying mass
      – Excess catecholamine secretion

Adrenal Medulla: F344 Rat
Pheochromocytoma
Factors Influencing Incidence of Pheochromocytomas in the Rat

- Age
- Strain
  - Holtzman 0.5%
  - Wistar 67%
- Sectioning technic
  - Single vs. serial
  - 7.5% of adrenal volume is medulla
- Chronic Stress
  - Chronic renal and lung disease

Focal Medullary Hyperplasia

<table>
<thead>
<tr>
<th>INTERSPECIES COMPARISON OF PHEOCHROMOCYTOMAS</th>
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<tr>
<td><strong>RAT</strong></td>
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<tr>
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<td>INDUCING AGENTS</td>
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<td>CELL TYPE</td>
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*BASED ON UNSELECTED AUTOPSY SERIES
ADRENAL MEDULLA: NEOPLASMS

- Malignant Pheochromocytomas
  - Local invasion
  - Invasion into the posterior vena cava

**Differential Diagnosis of Adrenal Medullary and Cortical Tumors**

- Chromaffin Reaction
  - Dichromate fixative oxidizes catecholeamines to form brown-black pigment
  - Differentiate cortical and medullary neoplasms
Adrenal Medulla: Ganglioneuroma

Dual Differentiation ('Complex')
Pheochromocytoma & Ganglioneuroma

Differential Diagnosis of Adrenal Medullary and Cortical Tumors

- Laboratory Diagnosis
  - Hormone secretion may be episodic and variable
  - Urinary hormones or metabolites are more reliable indicators
    - Catecholamines
    - Vanillylmandelic acid (VMA) – major metabolite
    - Urinary metanephrines
    - Urinary homovanillic acid (HVA)