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Species specific adrenal toxicity investigation, a case study

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Case history:

- Cortical hypertrophy (Zona fasciculata) and degeneration associated with increased adrenal gland weight (>21% relative to body weight) at various doses/exposures and duration in Sprague Dawley Rats

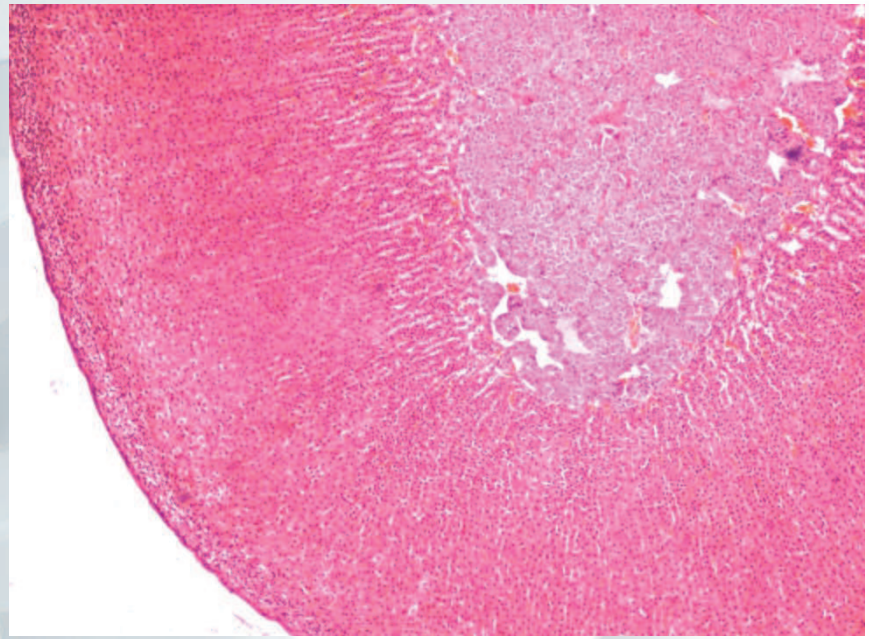
- No weight change in adrenal gland, no treatment related change in Cortisol and ACTH level and histological changes in adrenal gland in dogs at very high exposure in a short term study

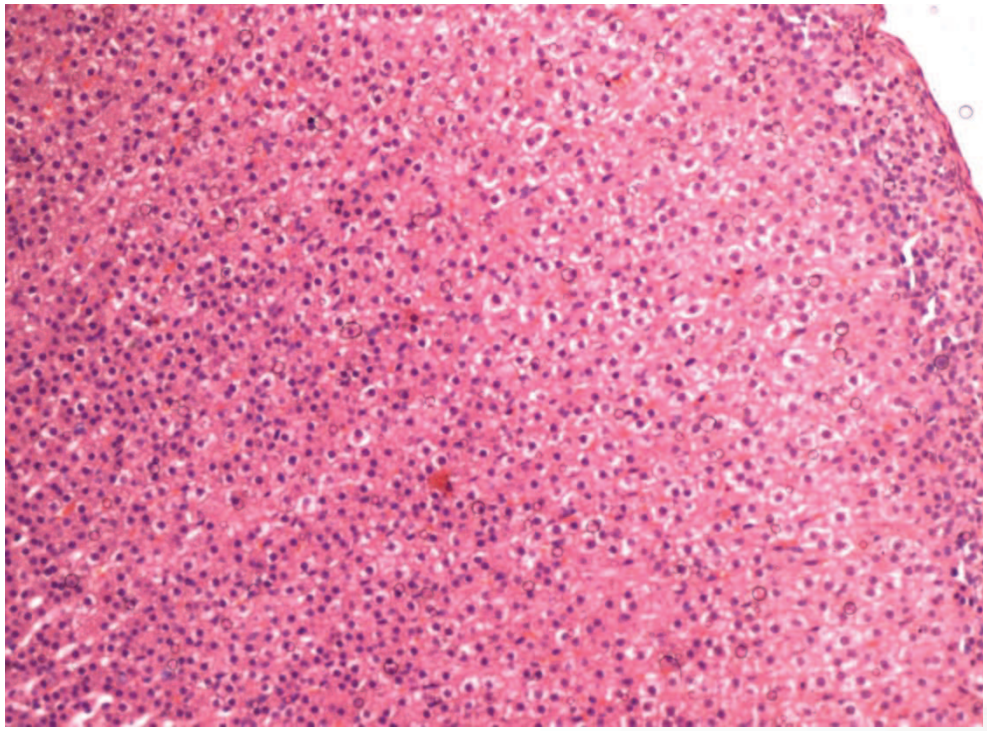
- Which is the right/relevant species to human from risk assessment perspective? Are there a way forward for developing a molecule?

Comparison of Toxicokinetic parameters from various studies

Species	Study Day	Doses (mg/kg/day)	Gender	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hr	Adrenal findings
Rat	28	0.3	Female	1,150	16,000	8	No
			Male	750	8,500	4	
		1	Female	1,790	21,250	4	
			Male	1,500	18,000	4	
		3	Female	6,900	82,000	4	Yes
			Male	7,700	106,000	8	
		10	Female	15,500	269,000	8	
			Male	11,500	181,000	4	
	14	10	Female	11,000	197,000	4	
			Male	8,500	125,000	8	
	7	30	Female	48,000	391,000	0	
			Male	21,000	333,500	1	
		100	Female	41,500	745,000	6	
			Male	34,500	500,000	6	
		250	Female	61,500	1,158,000	6	
			Male	37,000	678,000	8	
Dogs	7	1	Male	43,300	124,000	4	No
		10		491,500	2,350,000	2	

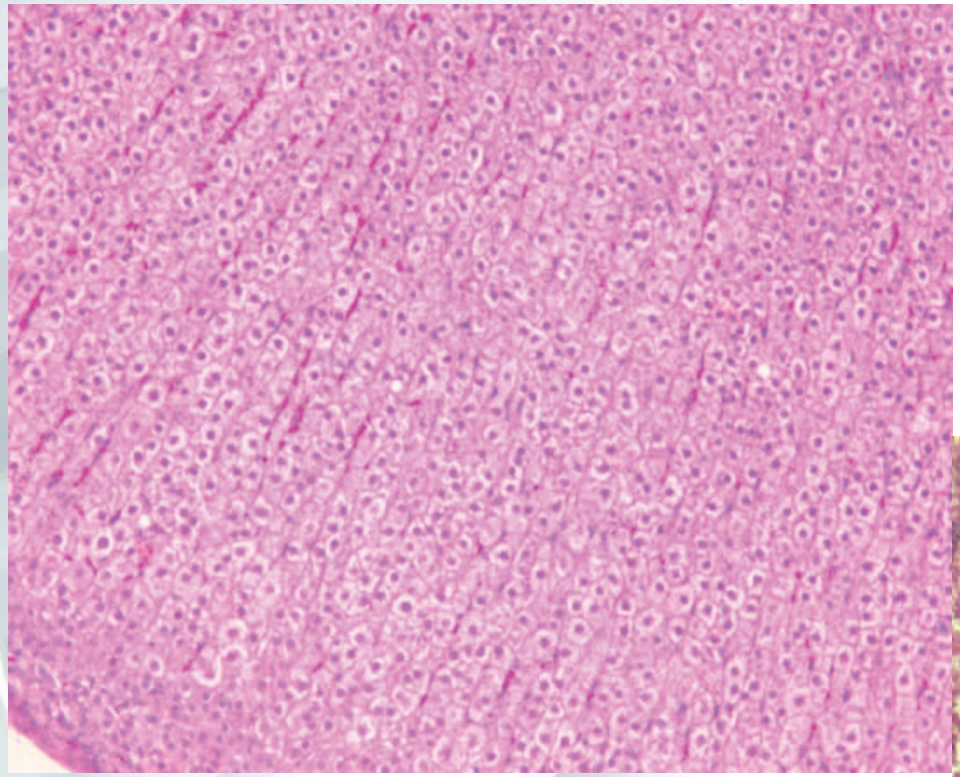
Microscopic changes

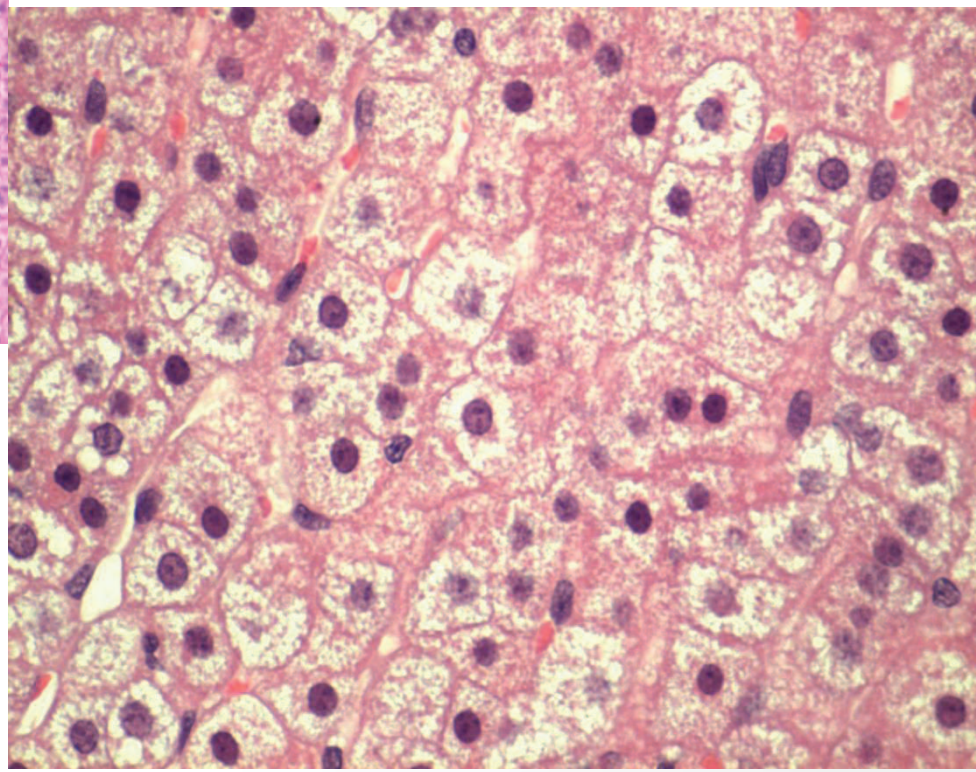




Normal structural and architecture of Adrenal gland (H&E, 4x and 10x)

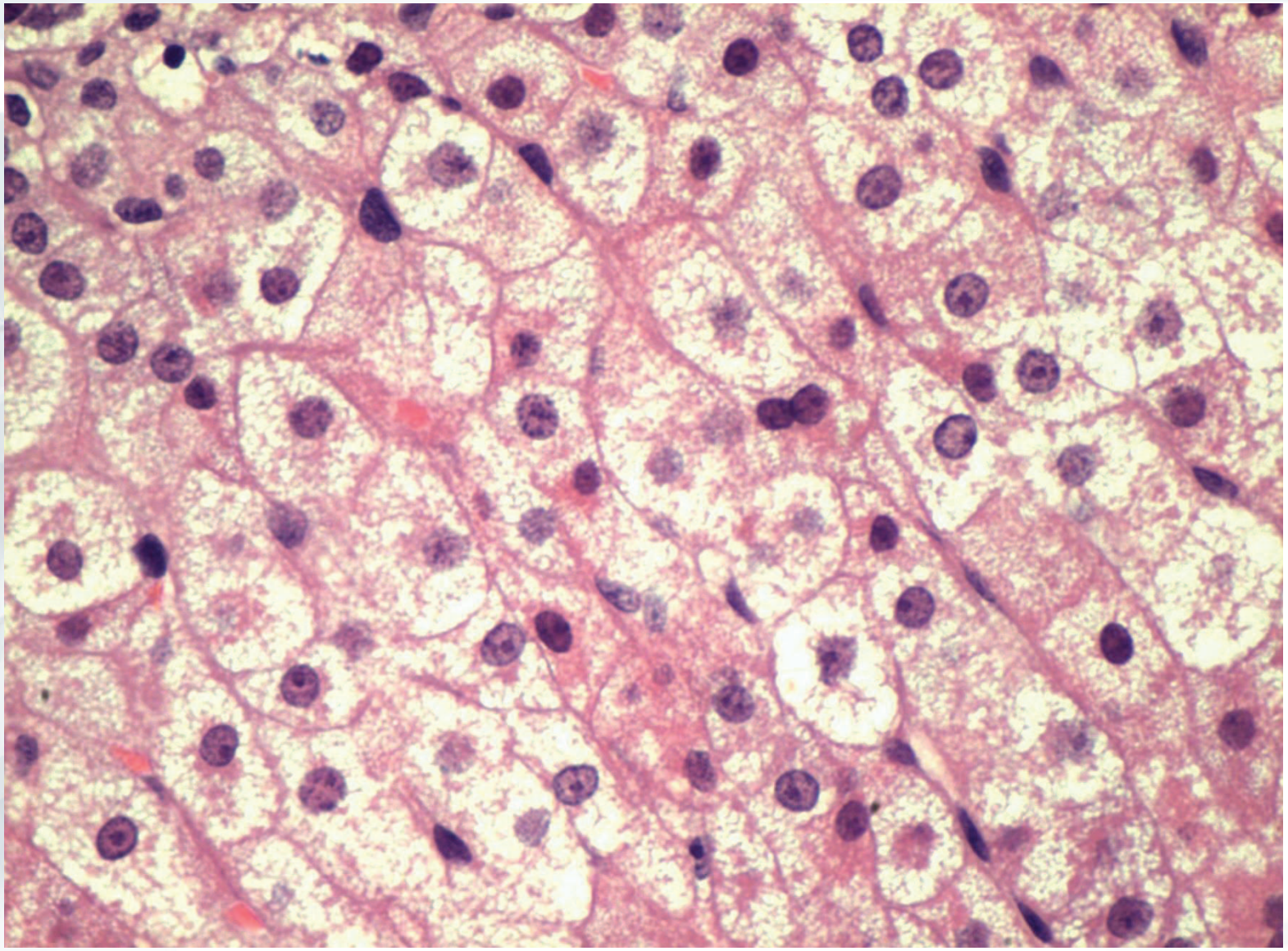
Microscopic changes





Cortical hypertrophy and hydropic degeneration (H&E, 10 and 40x)

Microscopic changes



Cortical hypertrophy and hydropic degeneration (H&E, 40x)

Significance or Safety concern of the lesion

- Akana *et al. (1983)* report that **adrenal hypertrophy is induced in rats by only 3 days of treatment with the steroidogenesis inhibitors** aminoglutethimide, cyanoketone or metyrapone. Aminoglutethimide has also produced **serious unpredicted clinical adrenal insufficiency resulting in patient deaths via 'pharmacological' inhibition of CYP11B1** (Camacho *et al., 1967; Vermeulen et al., 1983).

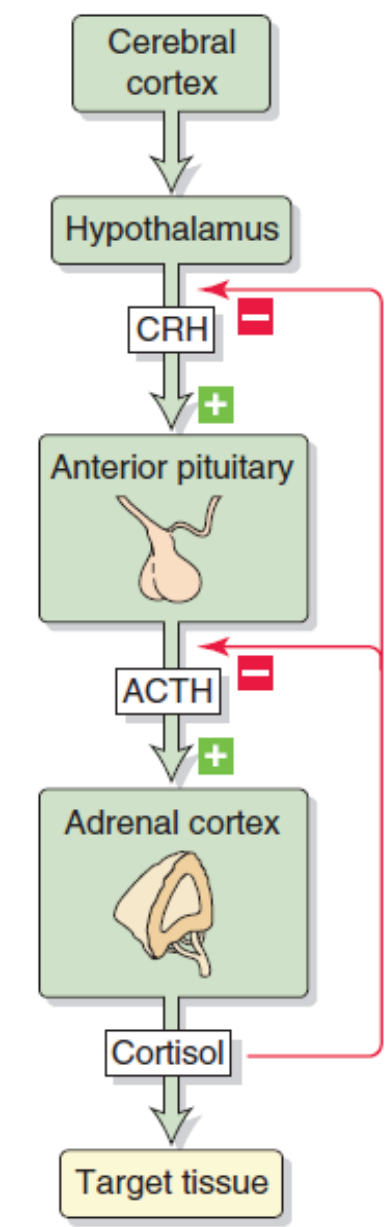
- A single acute dose of **etomidate** produces significant clinical adrenocortical insufficiency in human (Lundy *et al.,* 2007) due to selective CYP11B1 (11 Beta hydroxylase) enzyme inhibition.

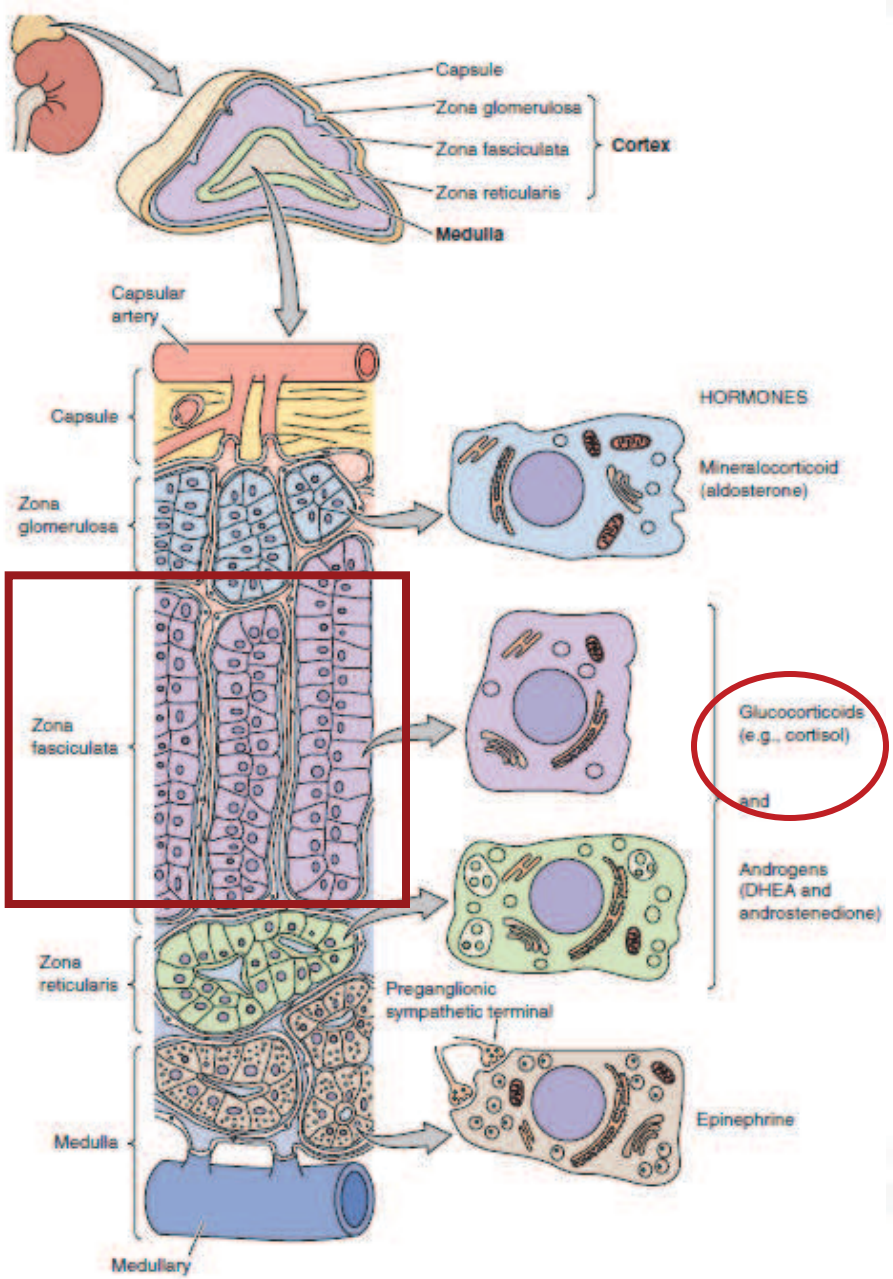
- The loss of adrenocortical steroidogenic capability, whether transiently pharmacological or due to a toxicopathological lesion, **is an adverse effect of toxicological significance and has serious implications for risk assessment. Even transient, reversible 'pharmacological' suppression of adrenocortical function can be a significant and dangerous adverse effect (Philip Harvey, 2010) .**

Investigational approach

- Location of the lesion and its functional consequences
- Differentiation b/n stress and direct toxicity
- Pathway of steroidogenesis, their inhibition and species specific pathways
- Knockout models data
- Is the findings rat specific?
- *In vitro* steroidogenesis assay
- Role of metabolite, if any
- Selection of appropriate species and conduct a tox study

Investigational approach – Location of the lesion and its functional consequences





Investigational approach – Differentiation b/n stress and direct toxicity

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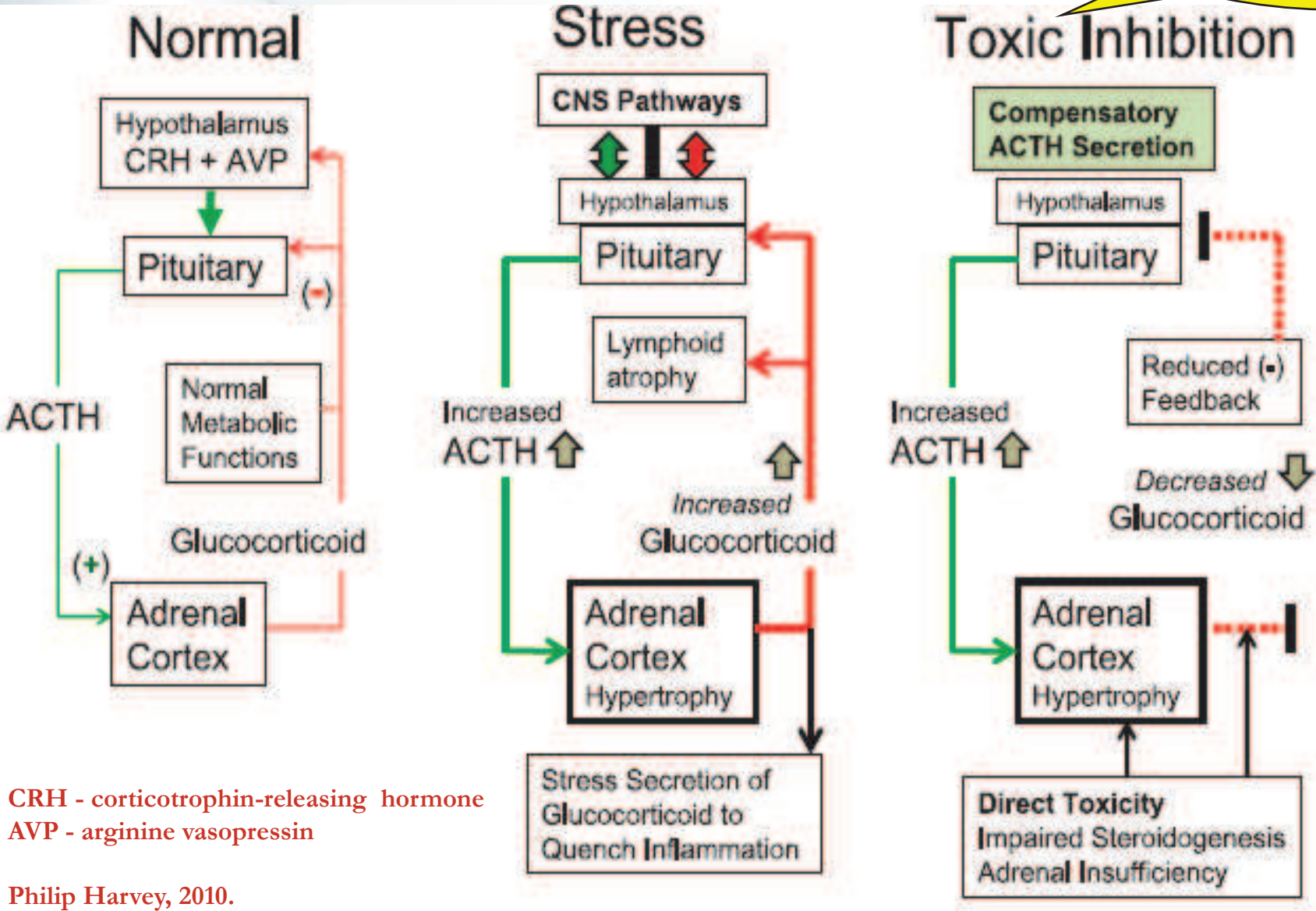
Candidate A

Hypothalamus-Pituitary-Adrenal (HPA) Axis:



CRH - corticotrophin-releasing hormone
AVP - arginine vasopressin

Philip Harvey, 2010.

Investigational approach – pathway of steroidogenesis, their inhibition and species specific pathways

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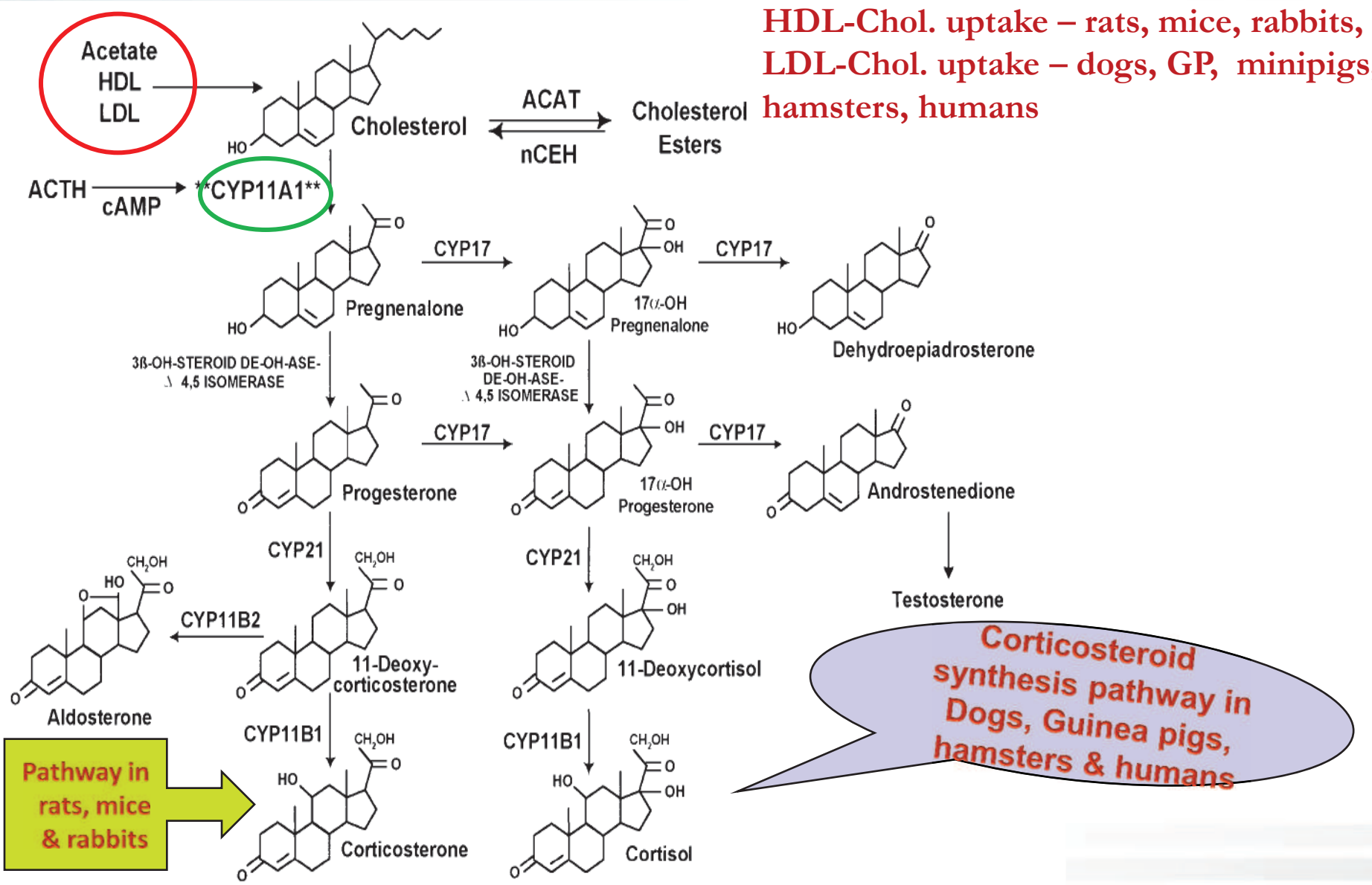
HDL-Chol. uptake – rats, mice, rabbits,
LDL-Chol. uptake – dogs, GP, minipigs, hamsters, humans



Thomas Rosol et al., 2001 and Gwynne et al., 1988

Steroidogenic inhibitors

Steroidogenic target	Compound
ACTH receptor	Aminoglutethimide
CYP11A1	Aminoglutethimide, dimethoate, Bromocriptine
CYP 17	Spironolactone, Ketoconazole, Flavonoids, PCB126, Thiazolidinediones-Pioglitazone, Salbutamol, Oestradiol, Bromophoneol, PCBs
CYP 21	RU 486, Ketoconazole, PCH26, PAHs
CYP 11B1	Metyrapone, Mitotane, Ethomidate, Ketoconazole, aminoglutethimide, Favonoids, PCB26, Efonidipine, Mibefradiol, Atrazine, simacine, Imidazole, vinclozalin, fenarimol , Amoxicillin, Cyprotene
CYP 11B2	Amidinohydrazones, PCB126, Fadrozole, PCBs, Efonidipine, Mibefradil, Amoxicillin, Erythromycin

Investigational approach – Knockout models data

- In adrenocortical cells, scavenger receptor class B, type I (SR-BI) retain HDL and are sites for the selective uptake of cholesteryl esters (CE) in rats and mice (Menno Hoekstra, 2009).

- **SR-B1 knockout mice (impaired adrenal HDL uptake) have lipid-depleted adrenocortical cells and adrenal hypertrophy that is mediated through ACTH, which is similar to changes seen in rats upon 'Candidate A' administration.**

Investigational approach – Is the findings rat specific?

- No lesions or significant changes in ACTH & Cortisol levels seen in dogs at ~120x than rat. It is known that dog is suitable species for understanding HPA mediated effect.
- No change in type of lesions following 7/14/28 days of 'Candidate A' administration at different dose levels in rats.

Possible explanations for rat specificity

- Lesion at relatively low exposure indicating 'alteration in some basic upstream event in the pathway' and no role of duration of administration.
- Differences in end product of corticosteroid as 'corticosterone' in rats vs Cortisol in dogs, human, hamsters etc.
- **In rat and mouse, HDL serves as the major cholesterol for adrenal steroidogenesis whereas LDL in hamsters, guinea pigs, dogs and humans (Thomas et al., 2001; David Spady et al, 1985).**
- Higher LDL levels in humans, dogs and hamsters provide more substrate for the LDL receptor-mediated uptake of **LDL cholesterol by the adrenals**.

Investigational approach – In vitro Steroidogenesis assay?

- **H295R steroidogenesis assay.**
  - Human adrenocortical tumour cell line and standard tool in endocrinology and recently entered the US EPA/OECD guidelines (2011) for endocrine function testing. Appropriately reflects steroidogenesis in humans than in rodent tissue.
  - Assay is validated and commonly used across industry especially for endocrine disruptors.
  - Cell viability and measurement of concentrations of Cortisol are the endpoint.
  - **Candidate A tested at various concentrations** (100 nM to 25 uM, based on Cmax and free fraction of compound), with and without human S9, steroidogenesis activator (Forskolin) and inhibitor (Perchloraz)
  - **Result**: No treatment related reduction in cortisol level up to 10 nM.

Investigational approach – Conduct a study in relevant species

Based on type of cholesterol used for steroidogenesis in animal species and human, absence of changes in dogs, KO animal data, in vitro steroidogenesis assay (H294R assay) results and irrelevance of mouse, a repeat dose study was conducted in Golden Syrian Hamsters at comparable exposure and duration to that performed in rat, and results showed;

- **No 'treatment related change in adrenal weight or histology' in Hamsters (LDL cholesterol uptake species, similar to human) indicating that 'Candidate A' is inhibiting HDL Cholesterol uptake (rats, mice & rabbits) in the steroidogenesis pathway, which is species specific effect.**

Comparison of toxicokinetic parameters from various studies

Species	Study Day	Doses (mg/kg/day)	Gender	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hr	Adrenal findings
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			Male	34,500	500,000	6	
		250	Female	61,500	1,158,000	6	
			Male	37,000	678,000	8	
Hamsters	14	10	Female	19,877	284,500	4	No
			Male	19,000	302,500	5	
		30	Female	42,000	593,500	4	
			Male	56,000	771,000	4	
Dogs	7	1	Male	43,300	124,000	4	No
		10		491,500	2,350,000	2	

Summary

- Species specific toxicological findings are not unusual, however proper interpretation of the result is necessary before concluding the fate of the molecule.
- A very strong one reason is sufficient to kill a molecule, however for the success of the molecule to drug, it has to meet all safety criteria, which is a challenge for Med Chemists/biologists/toxicologists etc.
- Selective HDL uptake inhibition in rat by Candidate A could be a unique case as there is no published literature on such mechanism.
- ONLY you can make the difference in evaluation of pathology data and identifying species relevance and extrapolation of findings to human.

Acknowledgements

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- Glenmark Research Centre
- STPI

Thank you