



SEEKING ANSWERS

Is atypical hyperadrenocorticism an early form of hyperadrenocorticism? Or is it possibly a separate disease that causes similar clinicopathologic changes?

ENDOCRINOLOGY

Behind the Scenes: Steroidogenesis and Thoughts on Atypical Hyperadrenocorticism

**MEET THE
AUTHOR**

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Mandy has over 17 years of experience as a Licensed Veterinary Technician (LVT). She obtained her certification as a Veterinary Technician Specialist (VTS) in Canine/Feline Clinical Practice in 2011 and is a Certified Veterinary Pain Practitioner (CVPP). She obtained a master's degree in veterinary biomedical science in 2018 through the University of Missouri. She is employed by Comanche Trail Veterinary Center in Liberty Hill, Texas, as the veterinary nursing supervisor. Her primary interest is internal medicine, with endocrinology as her passion, and is an active advocate for the advancement of the veterinary nurse profession.

Credentialed veterinary nurses are continuing to expand their roles in veterinary medicine. One overarching field of interest is endocrinology, which affects every discipline to some degree. As credentialed veterinary nurses, we serve on the front lines of patient care; therefore, expanding our knowledge and critical thinking skills with regard to the underlying intricacies of endocrinology can be instrumental in case management. My intent with this article is to highlight the challenges behind interpreting common clinicopathologic findings that resemble those of hyperadrenocorticism, a steroidogenic condition.

HYPERADRENOCORTICISM

Spontaneous hyperadrenocorticism is a common endocrinopathy of dogs. In those with this condition, hypercortisolism results when either a pituitary or an adrenal tumor disrupts the hypothalamic-pituitary-adrenal pathway. But before you test adrenal and pituitary function, you should evaluate your patient's history, physical examination findings, and biochemical changes. Common alterations associated with this disease include elevated alkaline phosphatase, hypercholesterolemia, proteinuria, minimally concentrated urine, polyuria/polydypsia, polyphagia, abdominal distention, and dermatologic abnormalities. When all arrows point toward hyperadrenocorticism, then low-dose dexamethasone suppression and/or adrenocorticotropic hormone (ACTH) testing is warranted. If your results do not support spontaneous hyperadrenocorticism, which often occurs, then you



should add atypical hyperadrenocorticism to your differential diagnoses. But how is atypical hyperadrenocorticism defined? Is it an early form of hyperadrenocorticism? Or is it possibly a separate disease that causes similar clinicopathologic changes?

Atypical Hyperadrenocorticism

The mechanism behind atypical hyperadrenocorticism is ambiguous; a multitude of possibilities may contribute to production and secretion of ACTH, cortisol, intermediate steroids, and/or other hormones, some of which may mimic cortisol's biochemical actions. The pathogenesis may be associated with a benign or malignant tumor, hyperplasia, steroid enzyme deficiencies, and/or aberrant expression of G protein-coupled receptors (GPCRs), all of which disrupt normal physiology and feedback mechanisms.

We will first discuss the normal physiologic process of steroidogenesis and then the abnormal processes involved with atypical hyperadrenocorticism.

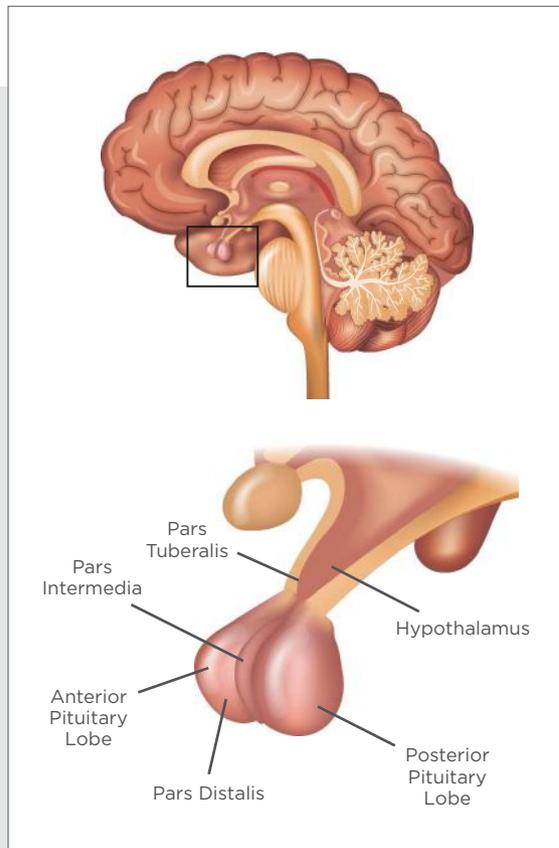


FIGURE 1. The pituitary gland.

NORMAL PHYSIOLOGY OF STEROIDOGENESIS

Normal physiologic control of adrenal steroids, including glucocorticoids, mineralocorticoids, and sex hormones, is regulated through diverse mechanisms and structures. The roles of these structures in steroidogenesis are discussed below.

Hypothalamus

The hypothalamus plays an integral role in maintaining homeostatic regulation and is positioned just above the pituitary gland (**FIGURE 1**). Its role is to receive and interpret afferent messages and respond to demands. Its intrinsic receptors measure hormone concentrations and glucose levels through the blood.¹ The paraventricular nucleus of the hypothalamus has numerous glucocorticoid receptors that mediate the synthesis and release of corticotropin-releasing hormone through negative feedback mechanisms. The messages received stimulate factors carried through the hypothalamo-hypophyseal portal system and either inhibit or stimulate the release of hormones from the pituitary gland, in which specialized endocrine cell types produce and secrete tropic hormones (hormones relayed from one endocrine gland to another) and play a pivotal role in messaging specific endocrine glands to function.^{2,3}

Pituitary Gland

The pituitary gland is divided into the neurohypophysis and the adenohypophysis, which is further divided into the pars distalis, pars intermedia, and pars tuberalis. The pars distalis encompasses the bulk of the anterior pituitary and consists of the following cell lines: corticotrophs, gonadotropes, lactotropes, somatotropes, and thyrotropes.^{3,4} Corticotroph cells synthesize ACTH through a precursor prohormone molecule, POMC (proopiomelanocortin). To synthesize ACTH, POMC must be cleaved at specific sites by prohormone convertase 1.^{5,6} ACTH is then released and targets the adrenal cortex.

Adrenal Glands

The adrenal glands are composed of the adrenal cortex and the medulla. The cortex has 3 zones: the zona fasciculata, zona reticularis, and zona glomerulosa. The zona fasciculata and reticularis are controlled by ACTH, and the zona glomerulosa is controlled by



BOX 1

Glossary of Steroidogenic Enzymes

- **CYP11A1 (cholesterol side chain cleavage enzyme):** Enzyme in the mitochondria of tissue-specific cells of the zona fasciculata of the adrenal cortex. It catalyzes the conversion of cholesterol to pregnenolone, the first reaction in steroidogenesis.
- **CYP17 (17-alpha-hydroxylase/17,20-lyase):** Enzyme in the smooth endoplasmic reticulum of tissue-specific cells of the zona fasciculata and reticularis of the adrenal cortex. It is essential for glucocorticoid synthesis (FIGURE 3).¹² Through hydroxylation, it first catalyzes the conversion of pregnenolone and progesterone from within the zona glomerulosa to 17-alpha-hydroxypregnenolone and 17-alpha-hydroxyprogesterone, respectively, within the zona fasciculata. This intermediate steroid can then either continue through enzymatic reactions within the zona fasciculata to form the end product, cortisol, or it can cleave at carbon bond 17-20 to dehydroepiandrosterone and androstenedione within the zona reticularis.
- **3 Beta-HSD (3-beta-hydroxysteroid dehydrogenase):** Enzyme in the smooth endoplasmic reticulum of tissue-specific cells of all layers of the adrenal cortex. It catalyzes the conversion of pregnenolone to progesterone within the zona glomerulosa, 17-alpha-hydroxypregnenolone to 17-alpha-hydroxyprogesterone within the zona fasciculata and DHEA (dehydroepiandrosterone) to androstenedione within the zona reticularis. Note that the steroidogenesis inhibitor trilostane inhibits this enzyme, although it does not affect, or may even increase, levels of 17-alpha-hydroxyprogesterone.
- **CYP21 (21-hydroxylase):** Enzyme in the mitochondria of the zona glomerulosa and fasciculata that catalyzes the conversion of progesterone to 11-deoxycorticosterone within the zona glomerulosa (aldosterone pathway) and enzymatically converts 17-alpha-hydroxyprogesterone to 11-deoxycortisol within the zona fasciculata (cortisol pathway). Deficiency of this enzyme through gene defects results in reduced cortisol synthesis and continued stimulation of ACTH, leading to adrenal hyperplasia and subsequent increase of other adrenal steroids.
- **CYP11B1 (11-beta-hydroxylase cytochrome P450):** Steroidogenesis in dogs and humans is similar, but the gene for CYP11B1 in the human genome is not found in that of dogs.¹¹ Humans have both the CYP11B1 and CYP11B2 (aldosterone synthase) enzymes; whereas for dogs, only 1 (CYP11B1) has been discovered. Therefore, for dogs, CYP11B1 is the enzyme involved in the remaining cascade for synthesizing both aldosterone and cortisol in the zona glomerulosa and zona fasciculata, respectively.¹¹ Within the zona glomerulosa, it converts 11-deoxycorticosterone to corticosterone, then to 18-hydroxycorticosterone, and then to aldosterone. Within the zona fasciculata, it converts 11-deoxycortisol to cortisol. In patients with corticotroph adenomas, this cortisol metabolic enzyme is overexpressed.¹³
- **11-beta-hydroxysteroid dehydrogenase system:** This system uses the enzyme 11B-HSD1 to convert inactive cortisone to active cortisol and 11B-HSD2 to convert cortisol back to cortisone.¹⁴ Inhibition of 11B-HSD2 in patients with corticotroph adenomas (pituitary-dependent hyperadrenocorticism) increases cortisol levels, which increases negative feedback to allow for reduced ACTH synthesis and reduced cortisol production by the adrenal glands. Research into use of 11-beta-HSD1 inhibitors for treatment of pituitary-dependent hyperadrenocorticism is ongoing.

the renin-angiotensin system. After ACTH binds to the adrenal glands' GPCR, complex intracellular signaling begins, hydrolyzing cholesterol within the cytosol. This action allows for free transport of cholesterol to the mitochondria, where the cholesterol is then carried across the mitochondrial membrane by the steroidogenic acute regulatory protein to convert cholesterol to pregnenolone, the rate-limiting step in steroidogenesis.⁷

Cholesterol

Cholesterol is the precursor to steroid synthesis and is

available through endogenous (synthesis) and exogenous (dietary) mechanisms. The body requires a constant supply of cholesterol, which is regulated through negative-feedback mechanisms. Cholesterol is synthesized primarily within the liver but also at secondary sites. When cellular cholesterol levels decrease, formation of HMG-CoA (5-hydroxy-3-methylglutaryl-coenzyme A) reductase (the rate-limiting enzymatic reaction used to synthesize cholesterol within the cell) is stimulated.⁷ After synthesis in the liver, cholesterol is bound to low-density lipoproteins for plasma transport, targeting the adrenal cortex. As low-density lipoprotein targets

low-density and high-density lipoprotein receptors, it is then internalized through endocytosis, fuses with lysosomes, and is then hydrolyzed into free cholesterol or stored as cholesterol esters in lipid droplets. Free cholesterol is transported to the mitochondria by the steroidogenic acute regulatory protein to initiate steroidogenesis.⁸

The Process of Steroidogenesis

Steroidogenesis occurs through modification of the base structure of cholesterol, composed of 27 carbons. This modification produces and bioactivates a variety of other steroids. Which steroid is produced depends on cell signaling. Steroid hormones are not stored but are instead secreted immediately after biosynthesis, which is why cholesterol has to be readily available.⁹ When ACTH binds to the adrenocortical receptor (also known as the melanocortin-2 receptor) on the cell membrane, it activates adenylyl cyclase to increase levels of cyclic adenosine monophosphate (cAMP) within the cytosol (FIGURE 2). Through secondary messaging, cAMP activates protein kinases to phosphorylate enzymes required for cholesterol conversion and formation of specific steroids, all of which are determined through negative-feedback mechanisms.¹⁰ Enzymes responsible for production of steroids are from the family of cytochrome proteins (CYP) (TABLE 1).^{10,11} These enzymes are most commonly known for their roles in drug metabolism and detoxifying, but a subset of these enzymes is used for steroidogenesis (FIGURE 3).¹¹

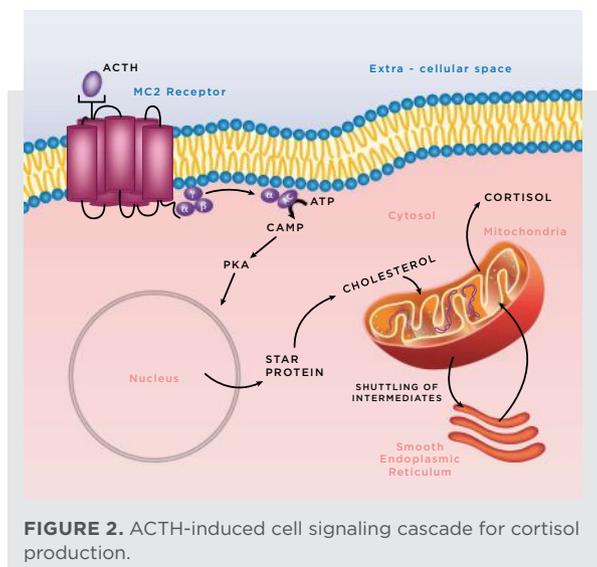


FIGURE 2. ACTH-induced cell signaling cascade for cortisol production.



The main contributors to hyperadrenocorticism are functional tumors, either benign or malignant, which disrupt normal metabolic regulation and cell differentiation.

ABNORMAL PHYSIOLOGY (DYSREGULATION) OF STEROIDOGENESIS

Many patients exhibit clinical signs that are associated with hyperadrenocorticism. Some cases are easily diagnosed and treated, but others remain uncontrolled even when ACTH test results are within normal limits. Other patients exhibit signs that resemble hyperadrenocorticism, but their adrenal and pituitary function test results are within normal limits. The physiology of the body is extremely complex; therefore,

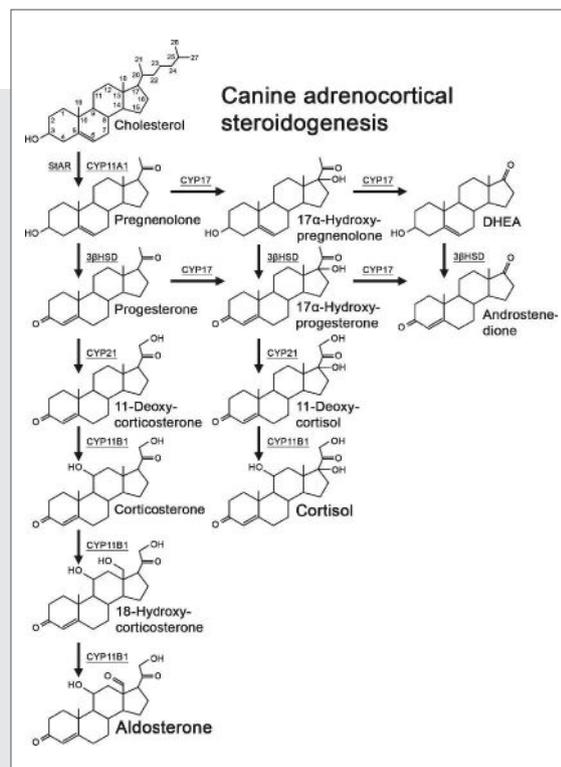


FIGURE 3. Steroid biosynthesis pathway.

the reason(s) for the disruption can be extremely difficult, if not impossible, to pinpoint. Ongoing research of atypical hyperadrenocorticism has led to more questions with few answers.

- 1. Laboratory Values:** Is it possible that the disease is in an early form and that the current reference ranges for common diagnostic testing are not sensitive enough?
- 2. Functional Tumors:** Do some tumors produce cortisol via non-ACTH stimuli?
- 3. Food:** Is food-induced hyperadrenocorticism (aberrant expression of gastric inhibitory polypeptide receptors in the adrenal cortex) more prevalent than realized?
- 4. Neuroendocrine Tumors:** Do some tumors produce ACTH ectopically?
- 5. Adrenal Glands:** Does intra-adrenal ACTH production play a role?

Laboratory Values

Re-evaluation of reference ranges for low-dose dexamethasone suppression and ACTH function test results has been discussed in light of the hypothesis that there is an early form of hyperadrenocorticism. One study showed that cortisol levels for atypical hyperadrenocorticism evaluated with low-dose dexamethasone suppression and ACTH testing were within the normal reference range but higher than those of healthy controls. Also, intermediate/precursor hormones alone do not cause the elevated alkaline phosphatase seen in dogs with hyperadrenocorticism and atypical hyperadrenocorticism.¹⁵ Therefore, the reason why patients with clinicopathological changes associated with hyperadrenocorticism have screening test results within reference ranges still needs to be identified.

Functional Tumors

The main contributors to hyperadrenocorticism are functional tumors, either benign or malignant, which disrupt normal metabolic regulation and cell differentiation. This disruption occurs in patients with spontaneous or atypical hyperadrenocorticism, but behavioral characteristics of the tumor may differ according to whether the tumor is an adenoma or carcinoma. Some mechanisms involved with tumorigenesis include the tumor's ability to become desensitized to normal negative feedback. In the dog, adrenal tumors (adenomas and carcinomas) do not upregulate genes encoded for steroidogenic enzymes.

TABLE 1

ECTOPIC RECEPTORS	EUTOPIC RECEPTORS
Gastric inhibitory polypeptide	Vasopressin (V1)
Beta-adrenergic	Luteinizing hormone
Vasopressin (V2, V3)	Serotonin (5-HT4)
Serotonin (5-HT7)	
Angiotensin II	
Glucagon	

The ability of an adrenocortical tumor to autonomously produce cortisol has been proposed to arise from the expression of aberrant receptors,¹⁶ associated with GPCRs. The expression of GPCRs stimulates steroidogenesis probably in the same manner as ACTH, in which after binding to its receptor, adenylyl cyclase is activated and stimulates the production of cAMP and activation of protein kinase A. This action enables phosphorylation of transcription factors that free up cholesterol and expresses steroidogenic enzymes to aid in steroidogenesis (adrenal carcinomas express a low level of the ACTH receptor gene).¹⁷ It is thought that both ectopic (abnormal site) and eutopic (normal site) GPCRs (**TABLE 1**) induce steroidogenesis through the same or similar cell signaling pathways. GPCR hormones such as gastric inhibitory peptide, vasopressin, and/or catecholamines have controlled cortisol secretion. Eutopic adrenal receptors include vasopressin (V1), luteinizing hormone, and serotonin (5-HT4). Ectopic adrenal receptors are gastric inhibitory peptide, beta-adrenergic receptors, vasopressin (V2, V3), serotonin, angiotensin II, and glucagon. When cortisol is produced by a non-ACTH stimulus, the negative-feedback mechanism is altered and endogenous ACTH stays low.¹⁸

Food

In patients with food-induced hyperadrenocorticism, gastric inhibitory polypeptide receptors are overexpressed within the zona fasciculata. As a meal is consumed, gastric inhibitory polypeptide (GIPR) is secreted and binds to the ectopic GIPR within the zona fasciculata, resulting in hypercortisolemia. Food-induced hyperadrenocorticism has been diagnosed in more than 30 human patients but in only 1 dog. That patient consumed its meal mid-day; therefore, because most patients eat morning and/or evening, traditional function testing did not capture the hypercortisolemia because of the timing of the test. Bilateral enlargement



Neuroendocrine tumors that ectopically secrete ACTH arise within the endocrine pancreas, adrenal medulla, intestines, thyroid gland, and lungs.



of this dog's adrenal glands suggested pituitary-dependent hyperadrenocorticism, but because of normal magnetic resonance images and low basal ACTH levels, exploration of food-induced hyperadrenocorticism was suggested.¹⁹

ACTH-Producing Neuroendocrine Tumors

Neuroendocrine tumors that ectopically secrete ACTH arise within the endocrine pancreas, adrenal medulla, intestines, thyroid gland, and lungs. Much like traditional adrenal hyperadrenocorticism, these ectopic ACTH-producing tumors fail to suppress cortisol during low-dose dexamethasone suppression testing but

with imaging there is no adrenal tumor identified, making the search for the clinicopathologic alterations challenging.²⁰ With regard to tumorigenesis, more and more literature is evolving on this pathogenesis, but more research is needed to understand the mechanisms and to develop more specific diagnostic options and more appropriate therapies to target the disease process more directly.

Adrenal Glands

Intra-adrenal ACTH expression and its paracrine/autocrine action (**FIGURE 4**) on the production of excessive cortisol, involving tumors and adrenal hyperplasia, have been evaluated in human adrenocortical cells. The chromaffin cells of the normal adrenal medulla secrete low levels of ACTH, which may play a role, via paracrine action, in the pathogenesis of hypercortisolism. In addition, a subpopulation of adrenocortical cells within various adrenal tumors and adrenal hyperplasia produce detectable levels of ACTH.²¹ The complexity of steroidogenesis involving intra-adrenal ACTH expression and the paracrine/autocrine mechanism involved with it is being studied with regard to human medicine, and it may be a contributor to atypical hyperadrenocorticism in our canine patients.

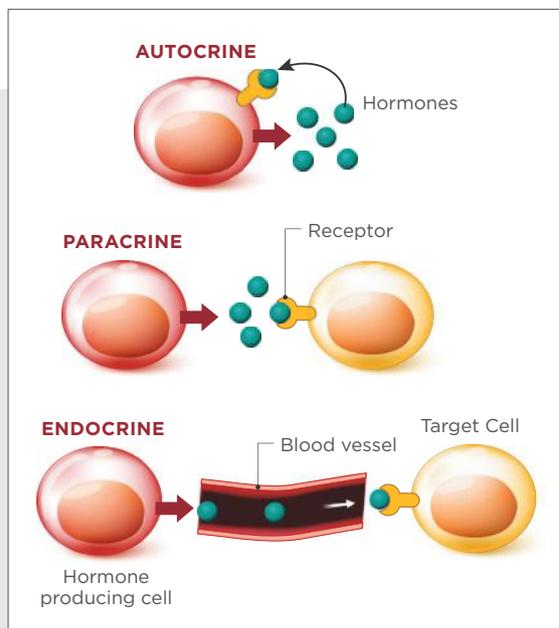


FIGURE 4. Cell signaling types and pathways: Autocrine describes self-signaling. Paracrine signaling communicates with nearby cells. Endocrine signaling describes a cell sending a signal through the bloodstream to communicate with a distant cell.

CONCLUSIONS

The hypothalamo-pituitary-adrenal axis is regulated, through negative feedback, by neuro-hormonal communications, intracellular messaging, and transcription. Dysregulation of the hypothalamo-pituitary-adrenal axis can be secondary to a variety of disease states, including genetic, autoimmune, cancer, and inhibitory and stimulatory effects, all with the ability to effect primary and secondary cascades. Overall, the ability of normal adrenal steroidogenic cells to grow, survive, differentiate, and function requires ACTH, vasopressin, angiotensin II, and insulin-like growth factors, and the ability of these substances to elicit proper cellular communication with appropriate follow-through of biochemical cascades to carry out the message. Continued research and possible identification of more appropriate diagnostics and targeted therapies may unravel some of the mysteries behind the clinical manifestations that develop in our patients with atypical hyperadrenocorticism. **TVN**



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