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Testing the Endocrine System for Adrenal Disorders and Diabetes Mellitus: It Is All About Signaling Hormones!

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For more information, please see the March 2012 companion article, "Testing the Endocrine System for Thyroid and Parathyroid Disorders: It Is All About Signaling Hormones!"

The endocrine system is complex and sometimes poorly understood. Although veterinary technicians often prepare and submit endocrine tests and care for patients with endocrinopathies, it may be difficult to understand what is happening in affected patients. This article unravels some of the mysteries of the endocrine system and highlights the need for testing to evaluate endocrine functions. A complete discussion of the endocrinopathies mentioned here is beyond the scope of this article. However, understanding endocrine testing can greatly enhance a technician's role in helping manage patients with endocrinopathies. The endocrine system comprises many glands and organs; this article focuses on testing for adrenal disorders and diabetes mellitus.

Adrenal Disorders

The adrenal glands are part of the interconnected hypothalamus, pituitary, and adrenal system, which is called the *hypothalamic-pituitary-adrenal* (*HPA*) *axis*. The HPA axis functions to ultimately produce cortisol. Each part of the axis, down to the adrenal glands, secretes a hormone that triggers release of another hormone from a different area within the HPA axis. When cortisol is secreted, the body reacts by ceasing to produce the previous hormones (**FIGURE 1**). The hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the pituitary to secrete corticotropin (also known as *adrenocorticotropic hormone*). In turn, corticotropin stimulates the adrenal glands to secrete steroids. The major steroids in the body are cortisol (an endogenous glucocorticoid) and aldosterone (an endogenous mineralocorticoid). When levels of endogenous steroids increase, their predecessor hormones decrease; this is called a *negative feedback loop*. When the cortisol level is high, CRH is inhibited and the corticotropin level decreases. Conversely, when the cortisol level is low, CRH is secreted and the corticotropin level increases.

Hypoadrenocorticism

Hypoadrenocorticism (also known as *Addison disease*) usually results from immune-mediated destruction of the adrenal glands. Clinical signs appear as affected patients become deficient in not only cortisol but also aldosterone.[1] Rarely, dogs develop secondary Addison disease due to failure of the pituitary to secrete corticotropin, resulting in adrenal failure. In addition, patients receiving antiadrenal therapy for hyperadrenocorticism can develop iatrogenic Addison disease. Adrenal dysfunction can be detected by measuring how well the adrenal glands respond to stimulation; therefore, the corticotropin stimulation test (also known as the *adrenocorticotropic hormone [ACTH] stimulation test*) is performed to diagnose this disorder.

Corticotropin is released from the pituitary during times of stress. When synthetic corticotropin or a gel form of corticotropin is administered during the corticotropin stimulation test, it stimulates the adrenal glands to produce cortisol. This response can be measured in an assay. Diagnosis of Addison disease involves measurement of an inappropriately low response to corticotropin stimulation. This definitively diagnoses hypoadrenocorticism because affected patients with a low basal cortisol level have no reserve when forced to respond to corticotropin stimulation.

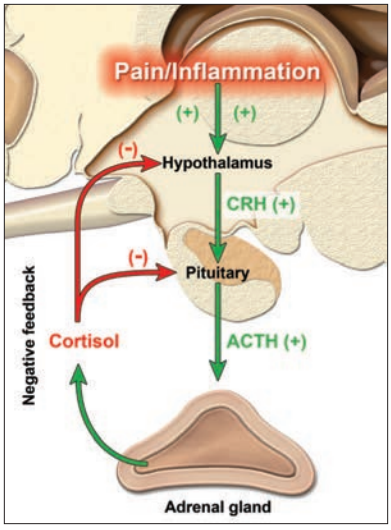

Illustration by Felecia Paras

Figure 1. Normal feedback loop of the HPA axis.

Hyperadrenocorticism

Hyperadrenocorticism (also known as *Cushing disease*)

involves overproduction of glucocorticoids and mineralocorticoids in the body. It is a complicated disease that can be caused by steroid therapy, a pituitary adenoma, or a functioning adrenal adenoma or adenocarcinoma. Most cases involve pituitary-dependent hyperadrenocorticism (PDH); fewer cases involve adrenal-dependent hyperadrenocorticism (ADH).

Several approaches may be used to test for hyperadrenocorticism. The differences are due to disagreement regarding which test should be used first and which tests should be used for confirmation. These tests have different sensitivity and specificity, so some are arguably better screening tests and others are better for confirming a result.

It may be beneficial to distinguish between PDH and ADH because a unilateral adrenal tumor may be surgically excised. The corticotropin stimulation test detects adrenocortical reserve and, therefore, sometimes reveals an elevated basal cortisol level and an exaggerated poststimulation cortisol level.[2] Eighty-five percent of dogs with PDH have exaggerated postcorticotropin results, while 55% of dogs with ADH have these results.[2]

Diagnosis of hyperadrenocorticism not only relies on excess cortisol production but also must show decreased sensitivity to exogenous glucocorticoid administration. When Cushing disease is suspected, a spontaneously obtained resting cortisol level is not diagnostic because many factors can elevate a single cortisol measurement. The primary test used to investigate the ability of the adrenal system to manage exogenous steroid administration is the low-dose dexamethasone suppression test (LDDST), which uses the potent steroid dexamethasone at a dose that elicits a diagnostic response without affecting the laboratory machine measurement of cortisol, as dexamethasone does not cross-react with the assay. In a healthy dog, because the corticotropin system is based on negative feedback, administration of exogenous glucocorticoids suppresses the corticotropin system, resulting in lowered cortisol measurements. However, in an affected patient, the "low" dose of steroids fails to suppress the HPA axis, resulting in elevated cortisol measurements. Therefore, LDDST results showing failure to suppress (i.e., the cortisol level is elevated) at 4 and 8 hours after administration are diagnostic of hyperadrenocorticism. This test is fairly accurate for diagnosing hyperadrenocorticism. In 65% of cases, the LDDST can differentiate between PDH and ADH through observation of the response (i.e., the cortisol level) at 4 and 8 hours after administration.[2] In some patients with PDH, cortisol production is temporarily suppressed after exogenous dexamethasone is administered. Thus, some suppression can be detected at 4 hours after administration, but the cortisol level (i.e., escape level) may be exaggerated again at 8 hours.[3] Patients with ADH will not have suppression at either time.

A noninvasive option to help diagnose hyperadrenocorticism is the urinary cortisol:creatinine ratio (UCCR) test. This test reflects several hours of cortisol production even though the UCCR sample is collected from one urination. This test is highly sensitive, making it a good screening test. Patients with normal UCCRs (reference range: <13) are unlikely to have hyperadrenocorticism. Typically, the client collects the UCCR sample by free catch at home. This can eliminate falsely elevated test results due to the stress of a veterinary visit. It is recommended to collect two samples on two consecutive mornings, with the second sample collected on the day of the veterinary appointment. The results are analyzed and averaged. An elevated urinary cortisol concentration in relation to the creatinine concentration suggests hyperadrenocorticism, but this should be confirmed with another test, such as the LDDST or corticotropin stimulation test. Stress or concurrent infection can raise the patient's cortisol level, leading to false-positive results. The UCCR test does not differentiate between PDH and ADH. Because test results from two consecutive days are averaged, diagnosis may be missed in patients with mildly elevated cortisol levels.

If initial diagnostic tests confirm a diagnosis of hyperadrenocorticism but cannot differentiate between PDH and ADH, a high-dose dexamethasone suppression test (HDDST) can be used. The HDDST should not be used as a screening test for hyperadrenocorticism; it should only be performed after a diagnosis has been confirmed through other appropriate testing. The clinical value of the HDDST lies in its ability (in many cases) to identify the type of hyperadrenocorticism a dog has, which can help clarify the most appropriate treatment options. This test administers 10 times more dexamethasone than the LDDST. The HDDST works on the premise that a high level of dexamethasone suppresses the corticotropin system in the presence of a pituitary-dependent tumor, based on negative corticotropin feedback, but does not suppress the corticotropin system in the presence of an adrenal tumor. With this test, if suppression is seen (i.e., cortisol levels are appropriately decreased from the baseline at 4 and 8 hours after administration), a diagnosis of PDH can be made. However, suppression does not occur in 25% of dogs with PDH; therefore, if suppression is not detected at 4 or 8 hours, the test cannot differentiate between PDH and ADH. In this case, the preferred method for differentiating between ADH and PDH is visualization of the adrenal glands by abdominal ultrasonography.

The plasma corticotropin level can also be used to differentiate between PDH and ADH. Patients with pituitary tumors have elevated corticotropin levels because their pituitary oversecretes corticotropin. Patients with adrenal tumors have decreased corticotropin levels due to negative feedback mechanisms. The plasma corticotropin level test is expensive and difficult to perform because of the requirements for sample handling. However, this test can be useful as a final laboratory test.

TABLE 1 summarizes the adrenal tests for hyperadrenocorticism.

Monitoring Diabetes Mellitus: Glucose Curves and Fructosamine Levels

Diabetes mellitus results from immune-mediated destruction or atrophy of β islet cells in the pancreas or from peripheral insulin resistance. Diabetes mellitus is diagnosed by detecting persistent hyperglycemia, glucosuria, and clinical signs. The glucose curve and fructosamine level tests are generally used to monitor insulin therapy and the blood sugar concentration.

The glucose curve is used to identify the highest blood glucose point, the lowest point (called the *nadir*), the difference between the high and low points, and the duration of action of insulin. The

Table 1. Differentiating Between PDH and ADH

	Test	Results Indicative of Hyperadrenocorticism	Basal Cortisol Level	Poststimulation Cortisol Level	Suppression at 4 Hours?	Suppression at 8 Hours?
Pituitary Dependent	UCCR test	Elevated cortisol level in relation to creatinine level	NA	NA	NA	NA
	Corticotropin (ACTH) stimulation test	Possibly elevated basal cortisol level; elevated poststimulation cortisol level	Possibly elevated	Probably elevated (85%): exaggerated response	NA	NA
	LDDST	Possibly elevated basal cortisol level; elevated poststimulation cortisol level	Possibly elevated	Probably elevated at 4 and 8 h	Yes: 65%	Maybe
	HDDST	Possibly elevated basal cortisol level; suppressed poststimulation cortisol level	Possibly elevated	Possibly elevated (25%)	Yes: 75%	Yes: 75%
	Corticotropin level test	Possibly elevated level	NA	NA	NA	NA
Adrenal Tumor	UCCR test	Elevated cortisol level in relation to creatinine level	NA	NA	NA	NA
	Corticotropin (ACTH) stimulation test	Possibly elevated basal cortisol level; elevated poststimulation cortisol level	Possibly elevated	Possibly elevated (55%): exaggerated response	NA	NA
	LDDST	Possibly elevated basal cortisol level; elevated poststimulation cortisol level	Possibly elevated	Elevated at 4 and 8 h	No	No
	HDDST	Possibly elevated basal cortisol level; elevated poststimulation cortisol level	Possibly elevated	Elevated at 4 and 8 h	No	No
	Corticotropin level test	Low level	NA	NA	NA	NA

glucose curve can be used to determine whether the insulin dose is appropriate. The nadir (i.e., peak insulin action) should be 100 to 125 mg/dL in dogs and 90 to 180 mg/dL in cats.[4] The glucose concentration should be 100 to 250 mg/dL in dogs for most of the day and 270 to 360 mg/dL in cats before insulin injection.[4] Unless glargine (a "peakless" insulin) is used, the blood glucose nadir should occur roughly halfway through the dosing period; therefore, if insulin is given every 12 hours, its activity should peak at ~6 hours after administration. However, blood glucose curve readings can be altered by the Somogyi effect, which begins when overtreatment with insulin results in a hypoglycemic episode (often at night). This causes the body to release counterregulatory hormones, including cortisol, glucagon, and epinephrine. These hormones maintain insulin resistance and glucose release and create a hyperglycemic effect. Thus, early-morning hyperglycemia may indicate a rebound from hypoglycemia earlier in the night.

Glucose curve abnormalities include an insufficient nadir and an inappropriate glucose differential (i.e., the difference between high and low concentrations). If the patient is receiving an inappropriately high level (>1.5 U/kg in dogs and cats[5,6]) of insulin, causes of insulin resistance (e.g., Cushing disease, obesity, chronic infection) should be investigated. If the patient is receiving a normal (≤1 U/kg) amount of insulin and is still displaying clinical signs or having unresponsive glucose curves, the dose should be increased, the duration of action increased by changing the type of insulin, the frequency increased (from once-daily to twice-daily dosing), or the Somogyi effect considered.

Fructosamine is a glycosylated protein that is irreversibly bound to glucose. Therefore, the glucose and fructosamine concentrations increase in proportion to each other. Because fructosamine degrades slowly, testing it can provide a long-term picture of glycemic control over a period of 1 to 3 weeks. Fructosamine

levels of 350 to 450 mg/dL indicate good glycemic control. Levels >500 mg/dL indicate prolonged hyperglycemia, and levels <350 mg/dL indicate episodes of hypoglycemia.[4]

Conclusion

The endocrine system is highly complex; therefore, critical thinking is required to interpret associated test results, which reflect multiple influences and feedback mechanisms. For veterinary technicians, a basic understanding of interpreting these test results is essential for patient care.

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1. **Which test evaluates long-term blood sugar concentrations?**
   - **a.** the LDDST
   - **b.** the corticotropin stimulation test
   - **c.** the fructosamine level test
   - **d.** the blood glucose curve

2. **Which test(s) identifies/identify whether a patient's cortisol level is suppressed by steroid administration?**
   - **a.** the LDDST
   - **b.** the LDDST and the HDDST
   - **c.** the corticotropin stimulation test, the LDDST, and the HDDST
   - **d.** none of the above

3. **What information can be gathered from a blood glucose curve?**
   - **a.** the duration of action of insulin
   - **b.** the nadir of blood glucose
   - **c.** the highest point of blood glucose
   - **d.** all of the above

4. **Which result of an LDDST is consistent with a diagnosis of Cushing disease?**
   - **a.** cortisol levels above reference range at 4 and 8 hours after administration
   - **b.** adequate cortisol suppression at 4 hours after administration, but cortisol elevation above reference range at 8 hours
   - **c.** adequate cortisol suppression at 4 hours and 8 hours after administration
   - **d.** a and b

5. **How much more dexamethasone is administered in an HDDST compared with an LDDST?**
   - **a.** two times as much
   - **b.** 10 times as much
   - **c.** 100 times as much
   - **d.** none of the above; there is no difference

6. **What is the major difference between the corticotropin stimulation test and dexamethasone suppression tests?**
   - **a.** The corticotropin stimulation test administers cortisol and measures the patient's cortisol level.
   - **b.** The corticotropin stimulation test administers corticotropin and measures the cortisol level; the dexamethasone suppression test does not.
   - **c.** Dexamethasone suppression tests administer a steroid; the corticotropin stimulation test administers corticotropin.
   - **d.** b and c

7. **Hypoadrenocorticism is defined as**
   - **a.** excess cortisol production.
   - **b.** deficient cortisol and, potentially, aldosterone production.
   - **c.** excess epinephrine production.
   - **d.** none of the above

8. **Which test is most often used to diagnose hypoadrenocorticism (Addison disease)?**
   - **a.** the LDDST
   - **b.** the HDDST
   - **c.** the UCCR test
   - **d.** the corticotropin stimulation test

9. **Which of the following statements is true?**
   - **a.** CRH is released from the pituitary, corticotropin from the adrenal glands, and cortisol from the hypothalamus.
   - **b.** CRH is released from the adrenal glands, corticotropin from the pituitary, and cortisol from the hypothalamus.
   - **c.** CRH is released from the hypothalamus, corticotropin from the pituitary, and cortisol from the adrenal glands.
   - **d.** none of the above

10. **Which test is effective for screening for Cushing disease?**
    - **a.** the fructosamine level test
    - **b.** the HDDST
    - **c.** the corticotropin suppression test
    - **d.** the UCCR test