

# Domperidone Causes an Increase in Endogenous ACTH Concentration in Horses With Pituitary Pars Intermedia Dysfunction (Equine Cushing's Disease)

Janice E. Sojka, VMD, MS; L. Paige Jackson, DVM; George Moore, DVM, PhD; and Margaret Miller, DVM, PhD

Horses with pituitary pars intermedia dysfunction (PPID) react to domperidone administration with an exaggerated increase in endogenous adrenocorticotropins (ACTH) concentrations. Specifically, a dose of 2.5 mg/kg domperidone orally resulted in an endogenous ACTH concentration in PPID horses that was  $2.9 \pm 0.68$  times baseline values at 4 h post-administration. Endogenous ACTH concentrations in horses without PPID do not exhibit a similar increase. The domperidone-response test represents a promising new evocative test that may prove useful in the diagnosis of PPID in horses. Authors' addresses: Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 (Sojka, Jackson); and Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 (Moore, Miller); e-mail: sojkaje@purdue.edu (Sojka). © 2006 AAEP.

## 1. Introduction

Equine pituitary pars intermedia dysfunction (PPID; Equine Cushing's Disease) is the most common endocrinopathy of horses and ponies,<sup>1</sup> and PPID is now known to be present in a relatively large number of aged horses.<sup>1,2</sup> The clinical signs associated with PPID have been well reviewed.<sup>3</sup> Briefly, they include hirsutism, laminitis, chronic infection, abnormal fat distribution, muscle wasting, weight loss, polyuria and polydipsia, and infertility. A recent report suggested that pituitary dysfunction is present in >70% of horses with idiopathic laminitis.<sup>4</sup>

The affected cell type in horses with PPID is the pars intermedia melanotroph. Melanotrophs do not respond directly to negative feedback through

the hypothalamus-pituitary-adrenal axis.<sup>5</sup> Rather, they possess D<sub>2</sub> receptors and are subject to the tonic inhibition of dopaminergic neurons that originate in the hypothalamus.<sup>6</sup> One hypothesis is that PPID arises secondary to dopaminergic neurodegeneration.<sup>6</sup> Dopaminergic tone on the melanotrophs of the pars intermedia decreases over time as the dopaminergic neurons die off. Without this inhibitory influence of dopamine, the melanotrophs hypertrophy and eventually undergo neoplastic change. Lending credence to this theory is the fact that the most effective treatment for PPID is pergolide, a dopamine agonist.<sup>7</sup>

The goal of this study was to determine if adrenocorticotropin (ACTH) secretion is altered by admin-

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## NOTES

Table 1. Endogenous ACTH (pg/ml) Before and After Domperidone Administration in Horses with PPID

Subject	Basal ACTH	+4 h	+8 h	4/0
1	30.8	128	67	4.2
2	188	580	826	3.1
3	49.6	167	169	3.4
4	271	566	410	2.1
5	37.8	96.9	70.5	2.6
6	22.3	50.1	64.4	2.2
7	25.8	72.4	38.7	2.8
8	77.1	223	110	2.9
Mean±SD	87.8±91.9	250.7±215.3	219.4±272.8	2.9±.68

Laboratory normal range for ACTH is 10–59 pg/ml.

istration of a dopamine-receptor antagonist. It was our hypothesis that the hypertrophied and/or neoplastic melanotroph cells in the pars intermedia of horses with PPID continue to be modulated to some degree by tonic dopaminergic inhibition from nerve cells originating in the hypothalamus. In addition, when dopaminergic inhibition is removed through administration of a D<sub>2</sub> receptor blocker such as domperidone, horses with PPID will respond with an exaggerated increase in secretion that can be detected by measuring serum concentrations of endogenous ACTH.

## 2. Materials and Methods

### Subjects

Sixteen horses were used in this study. Eight had either clinical or histopathologic evidence of a pars intermedia tumor or pars intermedia hypertrophy. The remaining eight horses had no clinical or histopathologic evidence of PPID. Clinical evidence of PPID included hirsutism, lack of shedding, abnormal fat deposition, and abnormal dexamethasone-response test results (return to baseline values within 24 h). Horses were not diagnosed with PPID if they did not have hirsutism, a history of improper shedding, or abnormal dexamethasone-response test results. The PPID horses were comprised of four mares and four geldings; their average age was 23.6 ± 6 yr (range = 17–37 yr). The breeds represented were Saddlebred (2), Quarter Horse (2), Arabian (2), Thoroughbred (1), and Pony of America (1).

The eight control animals consisted of five mares and three geldings; their average age was 18.5 ± 6.7 yr (range = 6–25 ± 8 yr). The breeds represented were Quarter Horse (4), Appaloosa (2), Saddlebred (1), and Belgian (1).

### Protocol

All testing was performed between September and January. Five milliliters of blood were collected into silicone coated EDTA tubes at 8:00 a.m. Immediately after the baseline sample was collected, 2.5 mg/kg domperidone<sup>a</sup> was given orally. Blood

was collected 4 and 8 h later. In all instances, the plasma was separated within 30 min of collection and frozen until assay.

Endogenous ACTH concentration was determined using a chemiluminescent immunoassay on an Immulite machine (DPC-Cirus)<sup>b</sup>. ACTH has been validated for the horse in this laboratory.

### Statistical Analysis

Using a paired t-test, the increase in plasma ACTH at 4 h (ratio of the +4 h concentration to the baseline endogenous ACTH concentration) was compared with PPID and control horses. Significance was set at  $p < 0.05$ .

## 3. Results

The effect of domperidone administration on horses with PPID and without PPID is given in Tables 1 and 2, respectively. The column labeled 4/0 represents the ratio of the +4 hour ACTH measurement divided by the baseline value. Thus, it represents the time change over baseline at 4 h.

In three of the PPID horses, the baseline endogenous ACTH was above our normal reference range (10–59 pg/ml); however, in all instances, there was a marked increase 4 and 8 h after domperidone administration. By contrast, none of the endogenous ACTH concentrations in the control horses were above the reference range at any point in time.

Because of the large variation in starting endogenous values, the average and standard deviations of ACTH concentrations in the PPID horses were difficult to compare with the control horses. For that reason, in addition to measuring absolute ACTH concentrations, the magnitude change from baseline was determined in all horses at the +4 h point. Comparing these values using a t-test, the PPID horses exhibited a significantly greater increase compared with control horses.

## 4. Discussion

All testing was performed between September and January. This is the time of the year when endogenous ACTH concentrations are highest.<sup>8</sup> Despite

Table 2. Endogenous ACTH (pg/ml) Before and After Domperidone Administration in Horses With No Evidence of PPID

Subject	Basal ACTH	+4 h	+8 h	4/0
9	20.5	24.6	28.7	1.2
10	21.3	42.1	28	1.9
11	16.1	28.2	15.9	1.7
12	24.9	50.4	47	2.0
13	15.3	28.9	24.8	1.9
14	17.2	21.9	28.3	1.3
15	19.8	37.6	36.7	1.9
16	43.6	28.9	27.8	.65
Mean±SD	22.3±9.1	32.8±9.7	29.6±9.04	1.56±0.48

Laboratory normal range for ACTH is 10–59 pg/ml.

this, five of eight horses with PPID had baseline ACTH concentrations within our laboratory's normal range. The effect of seasonal variation on diagnostic testing for PPID is still poorly understood, because the time of year during testing is not included in most of the literature describing PPID diagnostic protocols.

There is a long history in human and veterinary medicine that supports the superiority of evocative testing when evaluating suspected endocrinopathies.<sup>9</sup> Well-documented examples in human medicine include the use of pentagastrin to diagnose premalignant change in thyroid C cell hyperplasia<sup>10</sup> and the administration of the GH-releasing hormone to assess growth-hormone deficiency.<sup>11</sup> Testing is particularly helpful when evaluating animals early in the course of their disease or when assessing animals with occult disease.<sup>9</sup>

A common drawback for many of the currently described tests for PPID is that they do not test the pars intermedia cells directly. Diagnostic testing that involves measurement of either glucose or insulin (such as a fasting glucose and/or insulin measurement, the IV glucose tolerance test, insulin tolerance test, or the IV glucose tolerance test with insulin determinations) is merely verifying the presence of insulin resistance.<sup>12–14</sup> Although PPID may cause insulin resistance, other diseases (particularly the poorly defined equine metabolic syndrome), breed (ponies and draft horses are inherently more insulin resistant than light horses), and physiologic events (such as the stress caused by overnight fasting) may cause insulin resistance as well.<sup>15</sup>

Tests that involve dexamethasone or ACTH administration are primarily testing the hypothalamus-pars anterior-adrenal axis, and this is not primarily affected in horses with PPID.<sup>3,16</sup> In addition, results of the test vary with the season of the year, and testing in the autumn may lead to erroneous results.<sup>8</sup> A final drawback is that (whether it is deserved or not), dexamethasone has a reputation for causing laminitis in horses. Many owners and practitioners will not administer it to a horse that

either already suffers from laminitis or is deemed high risk.

Horses with clinical signs of PPID and abnormal dexamethasone response tests may have endogenous ACTH values in the normal range. Thus, there is possibly a high percentage of false negatives with this test. This was discovered in the current study, because five horses with PPID diagnosed by other means had baseline ACTH values within the normal range.

Of the currently described tests, only the thyrotropin-releasing hormone (TRH) response test is hypothesized to evoke a direct response from the pars intermedia cells.<sup>1,17</sup> However, this test may be prone to a high percent of false positives, because other factors, such as the stress of transport and venipuncture, may cause transient increases in cortisol. Additionally, TRH is available sporadically and can be extremely expensive.

Domperidone is a synthetic benzimidazole compound that is a specific dopamine receptor (D<sub>2</sub>) antagonist, but, unlike the D<sub>2</sub> antagonists metaclopramide and cisapride, it does not cross the blood-brain barrier.<sup>18</sup> It is currently used in equine medicine to prevent and treat the agalactia associated with ingestion of endophyte-infected fescue and subsequent decreased prolactin concentrations in mares.<sup>19</sup> A drawback when using this product is that it is not available in a parenteral form. Oral administration adds a layer of uncertainty to the diagnostic test, because a horse may ingest less than the desired amount because of poor oral-medication technique. Also, a horse suffering from delayed gastric emptying may not absorb enough of the drug to stimulate a response within 4 h, resulting in a false-negative test. Despite these drawbacks, domperidone was selected as the D<sub>2</sub> antagonist to investigate, because it is already marketed for use in the horse and has a wide margin of safety.

In summary, this study presents preliminary data that suggests that horses with PPID react to domperidone administration with an exaggerated increase in endogenous ACTH concentration. Horses without PPID do not exhibit a similar marked in-

crease. Specifically, a dose of 2.5 mg/kg domperidone orally resulted in an endogenous ACTH concentration that was  $2.9 \pm 0.68$  times baseline values at 4 h post-administration in horses with PPID. It should be noted that 2.5 mg/kg was selected empirically. Further research is needed to derive a dose-response curve in PPID animals; the optimal dosage and time interval need be established, and the domperidone-response test should be compared to other conventional methods of diagnosing PPID in horses.

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<sup>a</sup>Equidone, Equi-Tox, Inc., Central, SC 29630.

<sup>b</sup>Immulite, Diagnostic Products Corporation, Los Angeles, CA 90045