Effects of anesthesia with isoflurane on plasma concentrations of adrenocorticotropic hormone in samples obtained from the cavernous sinus and jugular vein of horses

James L. Carmalt VetMB, MVetSC

Tanya Duke-Novakovski BVMc, MSc

Harold C. Schott II DVM, PhD

Johannes H. van der Kolk DVM, PhD

Received June 14, 2015.
Accepted September 28, 2015.

From the Departments of Large Animal Clinical Sciences (Carmalt) and Small Animal Clinical Sciences (Duke-Novakovski), Western College of Veterinary Medicine, University of Saskatchewan, SK S7N 5B4 Canada; the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 (Schott); and the Department of Clinical Veterinary Medicine, Swiss Institute for Equine Medicine, Vetsuisse Faculty, University of Bern and Agroscope, 3012 Bern, Switzerland (van der Kolk).

Address correspondence to Dr. Carmalt (james.carmalt@usask.ca).

OBJECTIVE
To determine effects of anesthesia on plasma concentrations and pulsatility of ACTH in samples obtained from the cavernous sinus and jugular vein of horses.

ANIMALS
6 clinically normal adult horses.

PROCEDURES
Catheters were placed in a jugular vein and into the cavernous sinus via a superficial facial vein. The following morning (day 1), cavernous sinus blood samples were collected every 5 minutes for 1 hour (collection of first sample = time 0) and jugular venous blood samples were collected at 0, 30, and 60 minutes. On day 2, horses were sedated with xylazine hydrochloride and anesthesia was induced with propofol mixed with ketamine hydrochloride. Horses were positioned in dorsal recumbency. Anesthesia was maintained with isoflurane in oxygen and a continuous rate infusion of butorphanol tartrate. One hour after anesthesia was induced, the blood sample protocol was repeated. Plasma ACTH concentrations were quantified by use of a commercially available sandwich assay. Generalized estimating equations that controlled for horse and an expressly automated deconvolution algorithm were used to determine effects of anesthesia on plasma ACTH concentrations and pulsatility, respectively.

RESULTS
Anesthesia significantly reduced the plasma ACTH concentration in blood samples collected from the cavernous sinus.

CONCLUSIONS AND CLINICAL RELEVANCE
Mean plasma ACTH concentrations in samples collected from the cavernous sinus of anesthetized horses were reduced. Determining the success of partial ablation of the pituitary gland in situ for treatment of pituitary pars intermedia dysfunction may require that effects of anesthesia be included in interpretation of plasma ACTH concentrations in cavernous sinus blood. (*Am J Vet Res* 2016;77:730–737)

Adrenocorticotropic hormone is produced in the pars distalis and pars intermedia of the pituitary gland of horses. In clinically normal horses, most of the systemically circulating ACTH is from the pars distalis because ACTH from the pars intermedia undergoes further posttranslational modification by melanotrophs, which leaves approximately 2% of ACTH intact in healthy pars intermedia tissue as well as in adenoma tissue.[1] Adrenocorticotrophic hormone stimulates the adrenal glands to synthesize and release cortisol into the blood, which then acts on the hypothalamus and pituitary gland in a negative feedback manner to control circulating concentrations of cortisol and maintain homeostasis under various conditions.[2]

ABBREVIATIONS

P_{ETCO_2}	End-tidal partial pressure of carbon dioxide
POMC	Pro-opiomelanocortin
PPID	Pituitary pars intermedia dysfunction

Pituitary pars intermedia dysfunction is the most common endocrine disease of the equine species (it is also known as equine Cushing disease). There is mounting evidence that PPID develops as a consequence of progressive loss of hypothalamic dopaminergic innervation to the pars intermedia, which leads to hyperplasia and eventual adenoma formation of pars intermedia melanotrophs.[1,3] Pars intermedia melanotrophs produce POMC, a propeptide of 241 amino acids that is subsequently cleaved into smaller peptides by prohormone convertase enzymes. These peptides include $ACTH_{1-39}$ (amino acids 138 to 176 of POMC) as well as other POMC-derived peptides. Dopamine released by hypothalamic neurons inhibits POMC production in clinically normal horses, and loss of this regulatory control leads to increased production and release of POMC-derived peptides, including ACTH, in PPID-affected

equids. Consequently, detection of elevated ACTH concentrations in venous blood is a commonly used test to support a diagnosis of PPID.[4-7]

Hormones from the pars distalis and POMC-derived peptides from the pars intermedia are secreted into the secondary plexus of the hypothalamic-hypophyseal portal system and subsequently flow into the cavernous sinus that lines the hypophyseal fossa of the sella turcica and surrounds the pituitary gland. This cavernous sinus blood contains high concentrations of hormones from the pars distalis and pars intermedia. Fortunately, access to the cavernous sinus of horses can be accomplished by insertion of a flexible catheter into a superficial facial vein, which was first described in 1987.[8] Since that initial report, this technique has been used in numerous studies for collection of cavernous sinus blood to more directly assess hormone release from the pituitary gland[1,8,a] as well as to measure cavernous blood temperature as an estimate of brain temperature in exercising horses.[9] Use of a discrete peak–detection method for ACTH concentrations measured in frequently collected equine cavernous sinus blood samples has revealed that ACTH is released in a pulsatile manner from the pituitary gland, with (mean ± SD) 10.2 ± 1.4 ACTH concentration peaks (pulses)/h in Standardbreds.[10] However, because cavernous sinus blood mixes with venous blood from other parts of the head during flow into the jugular veins, hormone concentrations decrease and pulsatility of hormone release may no longer be apparent in venous blood. Thus, measurement of ACTH concentrations in cavernous sinus blood provides a more direct assessment of pars distalis and pars intermedia activity and would also provide a more accurate assessment of changes in ACTH concentration attributable to treatments for PPID.

Anesthesia of horses is an inherently stressful procedure that can result in elevations of plasma ACTH and cortisol concentrations.[11] Halothane-induced anesthesia (without subsequent surgery) resulted in a dose-dependent stress response in horses that was attributed to cardiopulmonary depression.[11,12] Induction of anesthesia with xylazine and ketamine, followed by maintenance of anesthesia with halothane or isoflurane, also caused an increase in cortisol concentration that may have also been a consequence of cardiopulmonary depression.[13] Horses in which anesthesia was maintained with isoflurane were more hypercapnic, compared with horses in which anesthesia was maintained with halothane; however, horses of both groups were hypotensive.

During the past few decades, the treatment of choice for ACTH-dependent Cushing syndrome in people has changed from medical treatment to surgical intervention (ablation of hypophyseal adenomas).[14] Furthermore, intraoperative measurements of ACTH concentrations in samples obtained from both internal jugular veins provide information about completeness of partial ablation and, consequently, support intraoperative decision making in human patients with Cushing syndrome undergoing transsphenoidal surgery.[b] The syndrome in equids is distinct with regard to clinical, biochemical, and pathological features from the condition in dogs or humans.[15] For instance, Cushing disease is characterized by an ACTH-producing anterior corticotroph pituitary adenoma in humans,[16] whereas the lesion is found in the pituitary pars intermedia in horses.[1] Despite encouraging phase III clinical trials that have led to FDA approval of 2 agents for treatment of human patients with Cushing disease, no agent has yielded results comparable to those for resection.[17] Techniques for ablation of the pars intermedia are a possible alternative to medical treatment of PPID.[18] A number of steps will be required to develop appropriate techniques. Additional knowledge about factors that may affect cavernous sinus ACTH concentrations is needed. Specifically, manipulation of the pituitary gland in PPID-affected horses will likely be performed with the horses anesthetized; consequently, the effect of anesthesia on both the amount and pulsatility of ACTH release in clinically normal (primarily pars distalis origin) and PPID-affected (primarily pars intermedia origin) equids needs to be determined. This will allow assessment of effects of ablation to be evaluated separately from the effects of anesthesia.

The objective of the study reported here was to determine whether maintenance of anesthesia with isoflurane results in alterations in ACTH concentrations or pulsatility in cavernous sinus blood of clinically normal horses. The null hypothesis was that anesthesia maintained with isoflurane would not result in alterations of ACTH concentration or pulsatility in cavernous sinus blood of horses.

Materials and Methods

Animals

Six clinically normal horses (5 mares and 1 gelding; 4 Quarter Horses, 1 Thoroughbred, and 1 Arabian) were included in the study. Mean ± SD body weight was 470 ± 32 kg (range, 427 to 500 kg), and mean age was 12.2 ± 6.0 years (range, 7 to 25 years). Horses were housed in box stalls and provided hay and water ad libitum. Mean number of hours of daylight at the time of the study was 13.9 ± 3.1 hours (range, 9.4 to 16.8 hours). All procedures involving the animals were approved by the University of Saskatchewan Animal Research Ethics Board and were in compliance with the Canadian Council on Animal Care guidelines for humane animal use.

Experimental procedures

Horses were sedated for catheter placement (day 0) by use of a combination of detomidine hydrochloride[c] (3 mg) and butorphanol tartrate[d] (3 mg) administered via a jugular vein. The horses remained in a

standing position (restrained with a halter and lead rope placed around the neck, but horses were not placed in stocks) during the subsequent procedures. Skin overlying both jugular veins was clipped of hair and aseptically prepared. Hair immediately rostral to the masseter muscle mass on the lateral aspect of the left side of the face was clipped and the skin similarly prepared. The left facial vein was identified via palpation, and 12 mL of 2% mepivicaine[e] was infiltrated by use of a 20-gauge needle into the dermal and subdermal layers in the prepared area. Techniques were as described in another study.[8] Briefly, a vertical 6-cm incision was made through the skin overlying the vein. Subcutaneous tissues were carefully dissected to expose the facial vein, facial artery, and parotid salivary duct. The vein was isolated and elevated with a loop of 2-0 polydiaxonone[f] suture. A No. 11 scalpel blade was used to make a small puncture into the vein, and a 7F 110-cm Swan-Ganz catheter[g] was inserted. The catheter was advanced 30 cm (5 cm beyond the necessary location in the ventral cavernous sinus in case the horse dislodged the catheter overnight), and preplaced sutures were used to affix the catheter within the vein, thereby occluding the vein. The vein was then allowed to retract into the incision, which was closed with 2 layers (subcutaneous layer and skin) of suture. The remainder of the catheter was secured on the horse's head by use of cyanoacrylate glue, and the head was bandaged to protect the incision and catheter.

Correct position of the tip of the catheter in the ventral cavernous sinus was determined initially by radiographic confirmation and subsequently on the basis of a characteristic behavioral response (opening of the eyes, cessation of chewing activity, and a slight backward movement) during flushing of the catheter with heparinized saline (0.9% NaCl) solution. This response did not diminish over the study period. The catheter was flushed with 3 mL of heparinized saline solution every 8 hours to maintain patency. No abnormalities in behavior were detected, and there were no alternations in physical examination findings (temperature, pulse, or respiration rate) before or after catheter placement. Horses continued to eat and drink as usual.

The following morning (day 1), each horse was restrained in its box stall by use of a halter and lead rope. Feed was placed in a container positioned at head height to encourage the horses to remain still. A 3-way stopcock was placed on the external end of the Swan-Ganz catheter. The catheter was retracted slightly (approx 5 cm), and 12 mL of blood was obtained every 5 minutes for 1 hour (collection of the initial blood sample was designated as time 0). The same volume of blood was concurrently removed from the ipsilateral jugular vein at 0, 30, and 60 minutes. Thus, 13 cavernous sinus samples and 3 jugular vein samples were collected. Blood was placed into plastic tubes containing EDTA, stored on ice during the collection period, and then centrifuged at 1,942 × *g* for 15 minutes at 4°C. Plasma was decanted into microcentrifuge tubes and frozen at –80°C within 2 hours after blood collection.

On day 2, horses were anesthetized. A 14-gauge, 133-mm over-the-needle catheter[h] was inserted in the left jugular vein and used for administration of lactated Ringer solution (10 mL/kg/h) and drugs. Horses were sedated with xylazine hydrochloride[i] (1 mg/kg, IV), and anesthesia was induced by IV administration of a mixture of ketamine hydrochloride[j] (2 mg/kg) and propofol[k] (0.4 mg/kg). The trachea was intubated with a cuffed endotracheal tube (inside diameter, 26 mm), and the horse was hoisted onto a padded operating table and positioned in dorsal recumbency. The endotracheal tube was attached to a large animal circle breathing system, and the lungs were ventilated[l] with oxygen by use of a tidal volume of 10 mL/kg and respiratory rate adjusted to achieve P_{ETCO_2} of 45 ± 5 mm Hg.[19] Anesthesia was maintained with isoflurane[m] in oxygen administered with a precision vaporizer. Butorphanol tartrate (0.05 mg/kg, IV) was administered as soon as the horse was positioned on the operating table, and a continuous rate infusion of butorphanol (0.01 mg/kg/h) was administered throughout the anesthetic period by use of a computerized syringe pump. Dobutamine[n] was used to maintain mean arterial blood pressure > 70 mm Hg in all horses.

A side-stream gas analyzer[o] was used to measure P_{ETCO_2} and end-tidal partial pressure of isoflurane from a sampling port positioned in the Y-shaped connector of the breathing circuit. The gas analyzer also displayed oxygen saturation of hemoglobin for a pulse oximeter probe placed on the tongue. A 20-gauge, 48-mm over-the-needle catheter[p] was percutaneously placed into a facial artery for measurement of systemic arterial blood pressures; a transducer[q] positioned at the level of the shoulder was used to determine a 0 pressure point. The pressure waveform was transmitted through noncompliant tubing filled with saline solution and displayed on a physiologic monitor.[r] The physiologic monitor calculated the heart rate from the arterial pressure waveform and also displayed an ECG. Nasopharyngeal temperature was also measured in 4 horses by use of the same physiologic monitor.

One hour after the start of inhalation anesthesia, blood samples were collected in accordance with the protocol used on day 1 (this was at approximately the same time of day as for sample collection on day 1). At the end of the blood collection period, horses were euthanized by administration of an overdose of sodium pentobarbital.[s] The brain and pituitary gland were removed and examined, and no gross abnormalities (enlargement) of the pituitary gland were detected. The tip of the catheter (when a catheter was left in situ) was found to be in the appropriate location within the cavernous sinus.

Measurement of ACTH concentrations

Plasma ACTH concentrations were measured by use of a commercial sandwich assay.[20,t] Plasma sam-

ples were thawed, and plasma and a control sample (bilevel ACTH control module)[u] were maintained on ice until analysis. Analyses were performed over a 2-day period. Intra-assay and interassay coefficients of variation were 1.7% and 2.3% or 2.3% and 2.9% for control sera with mean ACTH concentrations of 30.4 pg/mL (n = 5) or 400.0 pg/mL (8), respectively. Limit of detection stated by the manufacturer was 9 pg/mL.

Statistical analysis

Data pertaining to horse (age, sex, breed, date of procedure, number of hours exposed to daylight, condition [awake vs anesthetized], site of blood collection [jugular vein vs cavernous sinus], time of blood sample collection, and plasma ACTH concentration) were recorded on a spreadsheet program[v] and subsequently transferred to commercially available statistical programs.[w,x]

Plasma ACTH concentrations were logarithmically transformed to establish a normal distribution, and generalized estimating equations that assumed a normal distribution and controlled for repeated observations on each horse were used to examine the effects of age, sex, breed, number of hours exposed to daylight, condition, site of blood collection, and time of blood sample collection as variables with a possible effect on logarithmically transformed plasma ACTH concentrations. All potential variables were initially screened by use of univariate analysis, and variables with a value of $P < 0.2$ were considered for final multivariate models.[21] All final models were built by use of a manual backward elimination technique. Variables that were not significant were assessed as potential confounders. When 2 or more variables had significant ($P < 0.05$) effects, biologically plausible 2-way interactions were assessed, with significant interactions retained in the final model. Model residuals were examined to detect outliers and influential observations.

Furthermore, the time series for ACTH concentrations (all cavernous sinus blood samples for each horse at each time point) before and during anesthesia were analyzed by means of an automated deconvolution algorithm. A convolution integral was used to distinguish the processes that yielded the overall temporal behavior of a hormone. These components consisted of the rate of hormone entry into, and the rate of hormone removal from, the bloodstream. Automated deconvolution is characterized as a multiparametric deconvolution-based hormone pulse identification method and considers 4 order-specific values (namely, hormone concentration, an estimate of the precision of the hormone concentration, time, and the number of sample replicates). Automated deconvolution is superior to discrete peak–detection methods for locating hormone secretion events because it provides crucial information about basal secretion, secretion-event pulse mass (pulse secretion), and elimination half-life.[22,y] Variables generated by use of automated deconvolution analysis were examined for normality by use of the Kolmogorov-Smirnov test and then evaluated by means of a paired *t* test.

Results

Physiologic data obtained from the horses during anesthesia were summarized **(Table 1)**. There was no significant effect of age ($P = 0.21$) or number of hours exposed to daylight ($P = 0.78$) on the overall plasma concentration of ACTH, as controlled for site of collection. Mean ± SD plasma ACTH concentration was significantly ($P < 0.001$) higher in cavernous sinus blood (499 ± 190 pg/mL) than in jugular vein blood (49 ± 28 pg/mL; **Table 2**).

Use of a discrete peak–detection method revealed a significant reduction in the mean ± SD ACTH concentration per time series (from 549 ± 888 pg/mL to 439 ± 668 pg/mL; $P = 0.044$) and in the area under the concentration curve (from 35.2 ± 58.0 ng/mL/h to 28.5 ± 43.4 ng/mL/h; $P = 0.046$) during anesthesia with isoflurane **(Table 3)**. There was 1 concentration peak/time series. Automated deconvolution analysis revealed a total of 4 secretion peaks (3 in horses that were awake and not anesthetized [ACTH half-life, 12.0, 9.2, and 2.4 minutes] and 1 in a horse during anesthesia with isoflurane [ACTH half-life, 0.3 minutes]).

Table 1—Mean ± SD values for physiologic data obtained during anesthesia of 6 clinically normal horses.

	Time after induction of anesthesia (min)									
Variable	**30**	**40**	**50**	**60***	**70**	**80**	**90**	**100**	**110**	**120**
Mean arterial blood pressure (mm Hg)	64 ± 14	72 ± 10	71 ± 6	72 ± 17	70 ± 13	71 ± 6	74 ± 8	67 ± 8	71 ± 9	69 ± 7
Heart rate (beats/min)	37 ± 7	34 ± 5	33 ± 5	33 ± 2	34 ± 3	34 ± 4	36 ± 8	37 ± 8	37 ± 9	38 ± 12
P_{ETCO_2} (mm Hg)	48 ± 5	46 ± 3	46 ± 3	44 ± 3	44 ± 3	43 ± 2	43 ± 3	43 ± 2	44 ± 2	44 ± 2
Nasopharyngeal temperature (°C)†	36.3 ± 0.3	36.3 ± 0.4	36.2 ± 0.5	36.1 ± 0.5	36.0 ± 0.6	36.0 ± 0.5	35.8 ± 0.4	35.8 ± 0.5	35.7 ± 0.5	35.7 ± 0.5
Hemoglobin oxygen saturation (%)	96 ± 3	96 ± 3	97 ± 3	97 ± 2	96 ± 4	96 ± 4	96 ± 3	95 ± 3	96 ± 3	96 ± 3
End-tidal concentration of isoflurane (%)	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.3	1.6 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2

*Start of blood sample collection. †Represents results for only 4 horses.

Table 2—Mean ± SD plasma ACTH concentration (calculated before logarithmic transformation) for samples obtained from the cavernous sinus and ipsilateral jugular vein at various times from 6 clinically normal horses during a period when they were awake and during anesthesia with isoflurane.

Time (min)*	Condition	Site of blood collection	Mean ± SD concentration (pg/ML)
0	Awake	Cavernous sinus	276.2 ± 322.9
		Jugular vein	17.5 ± 9.7
	Anesthetized	Cavernous sinus	393.4 ± 585.1
		Jugular vein	90.6 ± 189.8
5	Awake	Cavernous sinus	136.2 ± 172.6
		Jugular vein	—
	Anesthetized	Cavernous sinus	1,247.3 ± 2,350.6
		Jugular vein	—
10	Awake	Cavernous sinus	308.6 ± 298.5
		Jugular vein	—
	Anesthetized	Cavernous sinus	257.1 ± 384.4
		Jugular vein	—
15	Awake	Cavernous sinus	508.0 ± 440.6
		Jugular vein	—
	Anesthetized	Cavernous sinus	363.7 ± 585.2
		Jugular vein	—
20	Awake	Cavernous sinus	599.2 ±733.4
		Jugular vein	—
	Anesthetized	Cavernous sinus	549.2 ± 1,089.1
		Jugular vein	—
25	Awake	Cavernous sinus	1,068.7 ± 2,164.5
		Jugular vein	—
	Anesthetized	Cavernous sinus	500.23 ± 1,021.8
		Jugular vein	—
30	Awake	Cavernous sinus	1,355.5 ± 3.001.4
		Jugular vein	25.3 ± 25.1
	Anesthetized	Cavernous sinus	572.5 ± 1,176.2
		Jugular vein	69.7 ± 69.8
35	Awake	Cavernous sinus	538.2 ± 1,019.8
		Jugular vein	—
	Anesthetized	Cavernous sinus	862.4 ± 1,934.7
		Jugular vein	—
40	Awake	Cavernous sinus	240.0 ± 327.9
		Jugular vein	—
	Anesthetized	Cavernous sinus	360.7 ± 710.6
		Jugular vein	—
45	Awake	Cavernous sinus	341.1 ± 420.9
		Jugular vein	—
	Anesthetized	Cavernous sinus	307.4 ± 411.1
		Jugular vein	—
50	Awake	Cavernous sinus	230.3 ± 235.0
		Jugular vein	—
	Anesthetized	Cavernous sinus	129.9 ± 169.8
		Jugular vein	—
55	Awake	Cavernous sinus	286.6 ± 483.2
		Jugular vein	—
	Anesthetized	Cavernous sinus	82.4 ± 70.3
		Jugular vein	—
60	Awake	Cavernous sinus	1,446.0 ± 2,993.8
		Jugular vein	35.6 ± 40.9
	Anesthetized	Cavernous sinus	92.2 ± 53.9
		Jugular vein	53.9 ± 38.3

*Time at which the first blood sample was collected was designated as time 0; for anesthetized horses, time 0 was 60 minutes after induction of anesthesia.

— = Not applicable; sample was not collected.

Discussion

The effects of anesthesia on the stress response have been evaluated with plasma hormone concentrations, but effects on pulsatility of ACTH have not been evaluated. Analysis of results for the present study indicated that anesthesia with isoflurane led to a reduction in mean ACTH concentration in the cavernous sinus of horses and a decrease in the area under the concentration curve. These results may have been a function of an anesthesia-induced reduction in the half-life of ACTH, but further studies would be necessary to definitively determine this. The reduction in mean ACTH concentration during anesthesia differs from results of earlier studies in which inves-

Table 3—Variables determined by use of deconvolution analysis for 6 clinically normal horses during a period when they were awake and during anesthesia with isoflurane.

Variable	Awake	Anesthetized*	*P* value†
ACTH concentration (pg/mL)‡	549 ± 888	439 ± 668	0.044
AUC (ng/mL/h)‡	35.2 ± 58.0	28.5 ± 43.4	0.046
No. of secretion peaks	3	1	NS

*Samples were obtained from anesthetized horses beginning 1 hour after induction of anesthesia. †Values were considered significant at $P < 0.05$. ‡Value reported is mean ± SD.

AUC = Area under the curve. NS = Not significant ($P \geq 0.05$).

tigators detected an increase (by use of halothane or an anesthetic protocol similar to that of the present study)[11–13] or no change in ACTH concentration (by use of isoflurane-anesthetized horses).[23] Anesthesia typically results in hypercapnia, hypoxemia, hypotension, and hypothermia. These physiologic alterations can cause an increase in tone of the sympathetic nervous system and stimulate a stress response. In horses, a combination of these physiologic alterations may be necessary to increase stress hormone concentrations to a substantial concentration.[24]

In a previous study[25] that involved the use of pentobarbital in horses, preservation of arterial blood pressure may have decreased the stress response, compared with the stress response after the use of halothane. In the study reported here, arterial blood pressure was maintained by IV administration of fluids and dobutamine, and use of such treatment has been found to reduce cortisol release.[24] However, simply maintaining arterial blood pressure with dobutamine or methoxamine alone does not appear to completely eliminate the stress response.[24,26] A capnogram was used to guide ventilator settings for the lungs of the horses in the present study. Capnograms can underestimate the actual arterial carbon dioxide tension, and therefore these horses may have been considered mildly hypercapnic. However, mild or severe hypercapnia alone has not been found to increase cortisol concentration.[25,27,28] For the horses of the study reported here, pulse oximeter measurements were between 90% and 98%, but they were never < 90%. Pulse oximeters can underestimate the actual hemoglobin oxygen saturation in horses[29]; therefore, hypoxemia was unlikely. However, arterial blood gas analysis was not performed to confirm the data obtained with the capnogram and pulse oximeter. In the horses of the present study, body temperature measured in the nasopharyngeal area decreased over time. Hypothermia may initiate a stress response, but mild hypothermia can decrease the concentration of stress hormones in humans.[30]

Induction of anesthesia with xylazine and ketamine has not been found to increase concentrations of stress hormones in horses.[31] The cortisol concentration did not increase until 60 and 80 minutes after xylazine administration in ponies anesthetized with halothane and isoflurane, respectively.[13] The α_2–adrenergic receptor agonists suppress the stress response by decreasing sympathetic tone[13,32]; therefore, we decided to begin collection of blood samples 60 minutes after induction of anesthesia to ameliorate the substantial hypophyseal suppression that occurs when xylazine is administered as a premedicant. However, the effect of xylazine alone on cortisol or ACTH concentrations in horses has not been definitively determined. Detomidine decreased or did not change cortisol concentrations in horses,[33,34] and medetomidine or xylazine did not change cortisol concentrations in dogs.[35] Butorphanol was used in the study reported here as part of a concurrent study conducted to examine histamine release attributable to administration of opioids. Infusions of butorphanol have not been found to influence stress hormone concentrations in horses during anesthesia.[36] Propofol was used as an induction agent in combination with ketamine; it was considered unlikely that propofol influenced the results 60 minutes after induction with the low dose that was used. However, effects of propofol on stress hormone concentrations have not been confirmed in horses.[37]

Plasma ACTH concentrations were significantly higher in cavernous sinus blood than in jugular vein blood, which can be explained by a dilutional effect of venous blood draining into the jugular veins from other areas of the head and neck. The gradient in plasma ACTH concentration between the cavernous sinus and jugular veins can be > 33- to 50-fold in the equine species,[38] compared with a gradient of 10- to 20-fold (depending on the time point) for the horses of the study reported here. Comparison of results for the present study with results for the more commonly used discrete peak–detection methods revealed that the amount of ACTH secreted during the sample collection period was significantly reduced during anesthesia and that the number of concentration peaks was minimal before and during anesthesia.There is no doubt that the latter was attributable to differences in assay characteristics (limit of detection for the assay, 9 pg/mL) and the fact we used only a single replicate (measurement), which thereby indirectly influenced detection of secretion peaks. Furthermore, only a single secretion peak was detected in the horses during anesthesia, compared with 3 secretion peaks detected when the horses were awake, unsedated, and standing. These differences were also likely attributable to assay characteristics used for the automated deconvolution analysis.

It is important to mention that the present study was conducted to evaluate the effects of anesthesia on ACTH secretion in clinically normal horses. Given that there are differences in synthesis, posttranslational processing, and secretion of POMC-derived peptides from the melanotrophs of the pars intermedia and corticotrophs of the pars distalis, as well as between horses with PPID and clinically normal horses, it would be prudent to perform a similar study with PPID horses to determine whether the outcomes differ.

As mentioned previously, there are substantial differences in the anatomy and pathophysiology of horses with PPID and humans with Cushing disease. In the authors' experience, a direct surgical approach in horses is complicated by the proximity of the optic chiasm for a sphenopalatine sinus approach, the depth of tissue in a ventral basisphenoid slot osteotomy approach, and technical challenges for a myeloscopic approach. There is an increase in the use of interventional radiographic techniques for intravascular treatment of a variety of neoplasms and developmental or acquired abnormalities in humans and small animals. An intravascular approach to the pituitary gland is possible,[15] but further studies are necessary before this can be considered a viable treatment option. In addition, the relative inaccessibility of the pars intermedia (sandwiched between the pars distalis and pars nervosa) may pose problems with regard to limiting collateral damage during surgical interventions. To be confident of a treatment effect, other possible confounding factors such as pathophysiologic effects of anesthesia and anesthetic drugs must be considered. Results of the present study indicated that manipulation of the pituitary gland in situ for the treatment of PPID may mean that measurement of plasma ACTH concentration (especially if it is a minimal concentration) in cavernous sinus blood is not a reliable indicator of interventional success, and the ACTH concentration in cavernous sinus blood would have to be interpreted in light of the effects of anesthesia. Further studies are necessary to determine appropriate methods of assessing immediate effects of interventional treatments applied to a pituitary gland.

Acknowledgments

Supported by Equine Health Research Fund at the Western College of Veterinary Medicine.

The authors declare that there were no conflicts of interest.

Footnotes

a. Irvine CH, Hunn R. Long term collection of pituitary venous blood in the unrestrained horse (abstr). *N Z Med J* 1984;97:735.
b. Bons J, Cornips E, van Zwam W, et al. Intra-operative ACTH measurements: a way to more successful surgery for Cushing's disease (abstr). *NTKC Ned Tijdschr Klin Chem Labgeneesk* 2015;40:103.
c. Dormosedan, Pfizer Animal Health, Pfizer Canada Inc, Kirkland, QC, Canada.
d. Torbugesic, Ayerst Laboratories, Pierrefonds, QC, Canada.
e. Carbocaine, Pfizer Animal Health, Pfizer Canada Inc, Kirkland, QC, Canada.
f. PDS II, Ethicon US LLC, Cincinnati, Ohio.
g. Edwards Lifesciences LLC, Irvine, Calif.
h. BD Angiocath, BD Infusion Therapy Systems Inc, Sandy, Utah.
i. Rompun, Bayer Healthcare, Mississauga, ON, Canada.
j. Vetalar, Bioniche Animal Health, Belleville, ON, Canada.
k. Propoflo, Abbott Laboratories, Saint-Laurent, QC, Canada.
l. Dräger AV, anesthesia ventilator, North American Dräger, Telford, Pa.
m. Isoflo, Abbott Laboratories, Saint-Laurent, QC, Canada.
n. Dobutamine injection USP, Sandoz Canada Inc, Boucherville, QC, Canada.
o. POET IQ anesthesia gas monitor, Criticare Systems Inc, Waukesha, Wis.
p. BD Insyte, BD Infusion Therapy Systems Inc, Sandy, Utah.
q. Truwave disposable pressure transducer, Edwards Lifesciences LLC, Irvine, Calif.
r. PB240 operating room monitor, Medtronic, Minneapolis, Minn.
s. Euthanyl Forte, Bimeda-MTC Animal Health Inc, Cambridge, ON, Canada.
t. Siemens ACTH kit and Immulite 1000 Chemiluminescent System, Siemens Canada, Oakville, ON, Canada.
u. Bi-level ACTH control module, Siemens Canada, Oakville, ON, Canada.
v. Microsoft Excel, Microsoft Canada Inc, Mississauga, ON, Canada.
w. SPSS, IBM Canada Ltd, Markham, ON, Canada.
x. PULSE XP, Pulse Analysis Software, Charlottesville, Va.
y. AutoDecon Software.zip. Available at: www.researchgate.net/publication/262566415_AutoDecon_and_Pulse_XP_Software. Accessed Jan 1, 2015.

References

1. Millington WR, Dybdal NO, Dawson R Jr, et al. Equine Cushing's disease: differential regulation of β-endorphin processing in tumors of the intermediate pituitary. *Endocrinology* 1988;123:1598–1604.
2. Alexander SL, Irvine CHG, Donald RA. Dynamics of the regulation of the hypothalamo-pituitary-adrenal (HPA) axis determined using a nonsurgical method of collecting pituitary venous blood from horses. *Front Neuroendocrinol* 1996;17:1–50.
3. McFarlane D. Equine pituitary pars intermedia dysfunction. *Vet Clin Equine* 2011;27:93–113.
4. van der Kolk JH, Wensing T, Kalsbeek HC, et al. Laboratory diagnosis of equine pituitary pars intermedia adenoma. *Domest Anim Endocrinol* 1995;2:35–39.
5. Couetil L, Paradis MR, Knoll J. Plasma adrenocorticotropin concentration in healthy horses and horses with clinical signs of hyperadrenocorticism. *J Vet Intern Med* 1996;10:1–6.
6. Lee ZY, Zylstra R, Haritou SJ. The use of adrenocorticotrophic hormone as a potential biomarker of pituitary pars intermedia dysfunction in horses. *Vet J* 2010;185:58–61.
7. McGowan TW, Pinchbeck GP, McGowan CM. Evaluation of basal plasma α-melanocyte-stimulating hormone and adrenocorticotrophic hormone concentrations for the diagnosis of pituitary pars intermedia dysfunction from a population of aged horses. *Equine Vet J* 2013;45:66–73.
8. Irvine CHG, Alexander SL. A novel technique for measuring hypothalamic and pituitary hormone secretion rates from collection of pituitary venous effluent in the normal horse. *J Endocrinol* 1987;113:183–192.
9. McConaghy FF, Hales JR, Rose RJ, et al. Selective brain cooling in the horse during exercise and environmental heat stress. *J Appl Physiol* 1995;79:1849–1854.
10. Alexander SL, Irvine CHG, Donald RA. Short-term secretion patterns of corticotropin-releasing hormone, arginine vasopressin and ACTH as shown by intensive sampling of pituitary venous blood from horses. *Neuroendocrinology* 1994;60:225–236.
11. Taylor PM. Equine stress responses to anaesthesia. *Br J Anaesth* 1989;63:702–709.
12. Luna SPL, Taylor PM. Pituitary-adrenal activity and opioid release in ponies during thiopentone/halothane anaesthesia. *Res Vet Sci* 1995;58:35–41.
13. Taylor PM. Stress responses in ponies during halothane or isoflurane anaesthesia after induction with thiopentone or xylazine/ketamine. *J Vet Anaesth* 1991;18:8–14.
14. Biller BMK, Grossman AB, Stewart PM, et al. Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. *J Clin Endocrinol Metab* 2008;93:2454–2462.
15. Miller MA, Pardo ID, Jackson LP, et al. Correlation of pituitary histomorphometry with adrenocorticotrophic hormone response to domperidone administration in the diagnosis of equine pituitary pars intermedia dysfunction. *Vet Pathol* 2008;45:26–38.
16. Cuevas-Ramos D, Fleseriu M. Treatment of Cushing's disease: a mechanistic update. *J Endocrinol* 2014;223:R19–R39.
17. Lau D, Rutledge C, Aghi MK. Cushing's disease: current medi-

cal therapies and molecular insights guiding future therapies. *Neurosurg Focus* 2015;38:1–10.
18. Sakes A, Arkenbout EA, Jelínek F, et al. Design of an endovascular morcellator for the surgical treatment of equine Cushing's disease. *Vet Q* 2015;35:165–169.
19. Wilson DV, Schott HC, Robinson NE, et al. Response to nasopharyngeal oxygen administration in horses with lung disease. *Equine Vet J* 2006;38:219–223.
20. Perkins GA, Lamb S, Erb H, et al. Plasma adrenocorticotropin (ACTH) concentrations and clinical response in horses treated for equine Cushing's disease with cyproheptadine or pergolide. *Equine Vet J* 2002;34:679–685.
21. Dohoo I, Martin W, Stryhn H. Model-building strategies. In: *Veterinary epidemiologic research*. 2nd ed. Charlottetown, PE, Canada: VER Inc, 2012;365–394.
22. Johnson ML, Virostko A, Veldhuis JD, et al. Deconvolution analysis as a hormone pulse-detection algorithm. *Methods Enzymol* 2004;384:40–54.
23. Dzikiti TB, Hellebrekers LJ, van Dijk P. Effects of intravenous lidocaine on isoflurane concentration, physiological parameters, metabolic parameters and stress-related hormones in horses undergoing surgery. *J Vet Med A Physiol Pathol Clin Med* 2003;50:190–195.
24. Taylor PM. Adrenocortical and metabolic responses to dobutamine infusion during halothane anesthesia in ponies. *J Vet Pharmacol Ther* 1998;21:282–287.
25. Taylor PM. The stress response to anaesthesia in ponies: barbiturate anaesthesia. *Equine Vet J* 1990;22:307–312.
26. Brodbelt DC, Harris J, Taylor PM. Pituitary-adrenocortical effects of methoxamine infusion on halothane anaesthetised ponies. *Res Vet Sci* 1998;65:119–123.
27. Taylor PM. Effects of hypercapnia on endocrine and metabolic responses to anaesthesia in ponies. *Res Vet Sci* 1998;65:41–46.
28. Khanna AK, McDonnell WN, Dyson DH, et al. Cardiopulmonary effects of hypercapnia during controlled positive pressure ventilation in the horse. *Can J Vet Res* 1995;59:213–221.
29. Koenig J, McDonnell WN, Valverde A. Accuracy of pulse oximetry and capnography in healthy and compromised horses during spontaneous and controlled ventilation. *Can J Vet Res* 2003;67:169–174.
30. Oak ZC, Young KC, Doo IL, et al. Intraoperative mild hypothermia does not increase plasma concentrations of stress hormones during neurosurgery. *Can J Anaesth* 2001;48:815–818.
31. Robertson SA. Some metabolic and hormonal changes associated with general anesthesia and surgery in the horse. *Equine Vet J* 1987;19:288–294.
32. Alexander SL, Irvine CHG. The effect of the alpha-2-adrenergic agonist, clonidine, on secretion patterns and rates of adrenocorticotropic hormone and its secretagogues in the horse. *J Neuroendocrinol* 2000;12:87–88.
33. Raekallio M, Leino A, Vainio O, et al. Sympatho-adrenal activity and the clinical sedative effect of detomidine in horses. *Equine Vet J Suppl* 1992;11:66–68.
34. Carroll GL, Matthews NS, Hartsfield SM, et al. The effects of detomidine and its antagonism with tolazoline on stress related hormones, metabolites, physiologic responses and behavior in awake ponies. *Vet Surg* 1997;26:69–77.
35. Ambrisko TD, Hikasa Y. Neurohormonal and metabolic effects of medetomidine compared with xylazine in Beagle dogs. *Can J Vet Res* 2002;66:42–49.
36. Dias BP, De Araujo MA, Deschk M, et al. Effects of an intravenous infusion of butorphanol in isoflurane-anesthetized horses on cardiorespiratory parameters, recovery quality, gastrointestinal motility and serum cortisol concentrations. *Acta Cir Bras* 2014;29:801–806.
37. Ruane-O'Hora T, Hall WJ, Markos F. The effect of alphaxalone–alphadolone, propofol, and pentobarbitone anaesthesia on the β-endorphin and ACTH response to haemorrhage in the pig. *Can J Physiol Pharmacol* 2011;89:521–526.
38. Luna SP, Taylor PM. Cortisol, peptides and catecholamines in cerebrospinal fluid, pituitary effluent and peripheral blood of ponies. *Equine Vet J* 1998;30:166–169.