

Pituitary-adrenal function in dogs with acute critical illness

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Objective—To evaluate pituitary-adrenal function in critically ill dogs with sepsis, severe trauma, and gastric dilatation-volvulus (GDV).

Design—Cohort study.

Animals—31 ill dogs admitted to an intensive care unit (ICU) at Washington State University or the University of Pennsylvania; all dogs had acute critical illness for < 48 hours prior to admission.

Procedures—Baseline and ACTH-stimulated serum cortisol concentrations and baseline plasma ACTH concentrations were assayed for each dog within 24 hours after admission to the ICU. The change in cortisol concentrations (Δ -cortisol) was calculated for each dog. Morbidity and mortality data were recorded for each patient.

Results—Overall, 17 of 31 (55%) acutely critically ill dogs had at least 1 biochemical abnormality suggestive of adrenal gland or pituitary gland insufficiency. Only 1 (3%) dog had an exaggerated response to ACTH stimulation. Dogs with Δ -cortisol \leq 83 nmol/L were 5.7 times as likely to be receiving vasopressors as were dogs with Δ -cortisol > 83 nmol/L. No differences were detected among dogs with sepsis, severe trauma, or GDV with respect to mean baseline and ACTH-stimulated serum cortisol concentrations, Δ -cortisol, and baseline plasma ACTH concentrations.

Conclusions and Clinical Relevance—Biochemical abnormalities of the hypothalamic-pituitary-adrenal axis indicative of adrenal gland or pituitary gland insufficiency were common in critically ill dogs, whereas exaggerated responses to ACTH administration were uncommon. Acutely ill dogs with Δ -cortisol \leq 83 nmol/L may be more likely to require vasopressors as part of the treatment plan. (*J Am Vet Med Assoc* 2008;233:87–95)

Acute illness can cause dramatic changes in endocrine function.¹ Activation of the HPA axis, as evidenced by an increase in secretion of ACTH and cortisol, is believed to be a vital part of the physiologic stress response and is essential for maintenance of homeostasis and adaptation during severe illness. Cortisol concentrations increase after stress, and the response is typically proportional to the magnitude of injury or disease process.² However, in human patients, sepsis and septic shock can be associated with increased serum cortisol concentrations and a loss of correlation between plasma endogenous ACTH and serum cortisol concentrations, with the endogenous ACTH concentration being surprisingly low.^{3,4}

Although absolute hypoadrenocorticism appears to be rare in critically ill human patients,^{5,6} several studies^{4,7,8} have provided proof for the syndrome of RAI in

ABBREVIATIONS

CRH	Corticotropin-releasing hormone
Δ -cortisol	Difference between baseline and ACTH-stimulated serum cortisol concentrations
GDV	Gastric dilatation-volvulus
HPA	Hypothalamic-pituitary-adrenal
ICU	Intensive care unit
RAI	Relative adrenal insufficiency

septic human patients, as evidenced by baseline plasma cortisol concentrations that are high or within the reference range and a relatively poor response to ACTH stimulation. The incidence of RAI in critically ill human patients is variable and depends on the underlying disease and severity of illness. The overall incidence of

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RAI in high-risk critically ill patients (eg, those with hypotension, shock, or sepsis) is approximately 30% to 45%. The incidence increases with severity of illness (sepsis > elective surgery > ward admission), with an incidence of 25% to 40% for critically ill patients in general.^{6,9,10}

Lack of adrenal gland responsiveness in humans appears to be associated with increases in morbidity and mortality rates.^{4,7,8} In humans with critical illness, RAI appears to be a temporary condition caused by the severe illness itself, and adrenal gland function returns to normal after the patient recovers from the illness.^{6,8} However, the recognition of human patients with RAI may be crucial because inadequate adrenal reserve is associated with a poorer outcome and also because administration of low doses of glucocorticoids to patients with RAI appears to reduce morbidity and mortality rates, particularly in septic patients.^{6-8,11}

Less is known about adrenal gland dysfunction in critically ill veterinary patients. Research through experimentally induced sepsis and hemorrhagic shock suggests that there is RAI in domestic animals,^{12,13} and a small number of clinical studies^{14,15,a-d} have suggested that RAI or RAI-like syndromes exist in certain populations of veterinary patients. Therefore, we hypothesized that pituitary-adrenal insufficiency exists in some populations of critically ill dogs, such as those with acute sepsis. Furthermore, we hypothesized that patients with pituitary-adrenal insufficiency have higher morbidity (eg, increased duration in an ICU or hospital; development of hypotension) and mortality rates when compared with rates for patients that have normal adrenal gland and pituitary gland function.

The purpose of the study reported here was to evaluate pituitary-adrenal function in acutely critically ill dogs with sepsis and in dogs with other types of nonseptic critical illnesses (ie, severe trauma and GDV) that were likely to result in a similar degree of illness and to determine the incidence of pituitary-adrenal insufficiency in these populations. Another purpose of the study was to compare the morbidity and mortality rates of dogs with and without pituitary-adrenal insufficiency.

Materials and Methods

Dogs—Thirty-one critically ill dogs admitted to the ICUs at Washington State University or the University of Pennsylvania with 1 of 3 conditions (sepsis, severe trauma, or GDV) were included in the study. Inclusion criteria for ill dogs included sepsis, GDV, or severe trauma of < 48 hours' duration before admission to the ICU and no prior history of illness. Dogs that had been administered drugs known to affect the HPA axis, such as glucocorticoids, etomidate, or ketoconazole, within the 4 weeks preceding the study were excluded. Dogs with known or suspected adrenal gland disease (ie, hypoadrenocorticism or hyperadrenocorticism) were also excluded.

Ten healthy dogs owned by students, staff, or faculty of the Washington State University Veterinary Teaching Hospital were also used solely to assess the correlation between baseline plasma endogenous ACTH and baseline serum cortisol concentrations. This group was enrolled as part of another study¹⁶ designed to deter-

mine the lowest of 5 doses of cosyntropin administered IV that would stimulate maximal cortisol secretion in healthy dogs. Dogs were deemed healthy on the basis of history and results of physical examination, a CBC, serum biochemical analysis, and urinalysis.

All aspects of the study for both ill and healthy dogs were approved by the institutional animal care and use committees at Washington State University and the University of Pennsylvania. Written consent of owners was obtained for all dogs enrolled in the study.

Group allocation—For the purpose of the study, sepsis was defined as a systemic inflammatory response to infection and was manifested by 2 or more of the following conditions: rectal temperature > 39.7°C (103.5°F) or < 37.8°C (100°F); heart rate > 160 beats/min; respiratory rate > 20 breaths/min or $Paco_2$ < 32 mm Hg; and WBC count > 12,000 cells, < 4,000 cells, or > 10% band cells.¹⁷ Infection was determined by positive results for bacterial culture of appropriate samples obtained from all septic dogs. All GDV dogs were identified on the basis of history and results of physical examination and survey abdominal radiography. Severe trauma dogs had blunt or penetrating traumatic injuries involving multiple organ systems and were identified on the basis of history and results of physical examination and other diagnostic tests deemed appropriate for each patient.

Blood collection and ACTH-stimulation protocol—In ill dogs, blood samples for analysis of hormone concentrations were collected via venipuncture or indwelling central or peripheral venous catheters within 24 hours after admission to the ICU. Blood was first collected for determination of baseline plasma endogenous ACTH and serum cortisol concentrations. For the 10 healthy dogs, baseline plasma endogenous ACTH and serum cortisol concentrations were assayed 5 times at 2-week intervals during a 10-week period.¹⁶ For all dogs, 2.0 mL of blood for determination of plasma endogenous ACTH concentrations was collected into tubes that contained EDTA and the proteinase inhibitor aprotinin (final concentration, 1,000 kallikrein inhibitor units/mL of blood).¹⁸ Tubes were gently inverted 3 times, and contents were rapidly transferred to a plastic tube. Plastic tubes were centrifuged for 10 minutes, and plasma was harvested, placed in another plastic tube, and frozen at -80°C until analysis. Three milliliters of blood for the determination of baseline serum cortisol concentrations was placed in a standard serum clot tube and allowed to sit for 20 minutes. Tubes were then centrifuged for 10 minutes, and serum was harvested, placed in another plastic tube, and frozen at -80°C until analysis.

After collection of baseline blood samples in ill dogs, the ACTH-stimulation test was then performed. Each ill dog received cosyntropin^e (5 µg/kg [2.3 µg/lb]; up to a maximum of 250 µg/dog) IV.^{16,19-21,f} Because a cosyntropin dose of 1 µg/kg (0.45 µg/lb) provides maximal adrenal stimulation and a similar response to a dose of up to 5 µg/kg,^{19,20} all dogs, even those weighing > 50.0 kg (110 lb; n = 3; 51.8, 52, and 53.3 kg [114.0, 114.4, 117.3 lb, respectively]) received a maximally stimulating dose. Cosyntropin was supplied as 250 µg of lyophilized powder in 2-mL vials. Each vial

of cosyntropin was reconstituted with 1.0 mL of sterile saline (0.9% NaCl) solution in accordance with the manufacturer's directions and was administered at this concentration (ie, 250 µg/mL). Freshly reconstituted and frozen-thawed cosyntropin were used to perform ACTH-stimulation tests. Vials of cosyntropin were reconstituted at the time of the ACTH-stimulation test, and the appropriate amount was administered IV to a specific dog. Any unused portion of reconstituted cosyntropin was placed in a plastic syringe, labeled with the date, and immediately frozen at -80°C . Reconstituted cosyntropin can be stored in plastic syringes for as long as 6 months at -20°C with no adverse effects on bioactivity.²² Subsequently, frozen cosyntropin was thawed and warmed to ambient temperature prior to IV administration. All frozen cosyntropin was used within 6 months after reconstituting and freezing.

Three milliliters of blood was obtained 60 minutes after administration of cosyntropin for the determination of ACTH-stimulated serum cortisol concentrations. All blood samples for determination of ACTH-stimulated serum cortisol concentrations were processed in the same manner as described for the baseline serum cortisol samples.

Hormone assays—Baseline and ACTH-stimulated serum cortisol concentrations were measured with a radioimmunoassay⁸ validated for use in samples obtained from dogs.²³ Intra-assay and interassay coefficients of variation for the assay were 5.1% and 10.3%, respectively.²³ For ill dogs, all samples for measurement of cortisol concentrations were assayed in duplicate in a single batch. For healthy dogs, samples for measurement of cortisol concentrations were assayed in duplicate in multiple batches; however, samples from each dog were assayed in a single batch when possible. After completion of all cortisol assays, the value for Δ -cortisol was calculated for each dog.

For healthy dogs, baseline plasma endogenous ACTH concentration was determined by use of an assay validated for use in samples obtained from dogs.^{24,h} Because the endogenous ACTH assay was no longer available for use on samples obtained from the ill dogs, baseline plasma endogenous ACTH concentration for ill dogs was determined by use of an immunoradiometric assayⁱ that used 2 antibodies (1 against the peptide sequence ACTH 1–16 and the other against the peptide sequence ACTH 24–39). Parallelism was determined by use of serial dilution and assay of 4 plasma samples from dogs; all dilutions yielded lines with slopes similar to that for a standard ($P > 0.05$; Student *t* test). Intra-assay and interassay coefficients of variation for the immunoradiometric assay were $< 15\%$ and $< 19\%$, respectively, for aliquots of plasma pools with approximately 34 and 210 pg of endogenous ACTH/mL. Sensitivity was 4 pg/mL. Finally, endogenous ACTH concentrations in plasma obtained from a dog given CRH (1 µg/kg, IV) had an expected rapid increase, whereas administration of dexamethasone (0.1 mg/kg [0.045 mg/lb], IV) resulted in nondetectable ACTH concentrations in samples obtained 4 and 8 hours after injection.

Furthermore, assay results were compared with those obtained by use of a previously validated ACTH immunoradiometric assay.^{24,h} The ACTH concentra-

tions were determined by use of both immunoradiometric assays for 22 plasma samples obtained from healthy dogs not included in the study. Samples had a wide range of ACTH concentrations (4 to 336 pg/mL). Analysis of plotted values yielded a line with the equation $y = 0.78x + 0.97$ pg/mL, where *y* is the assay result for the immunoradiometric assay described here, and *x* is the assay result for the previously validated immunoradiometric assay. The correlation coefficient was 0.95 ($P = 0.01$). A bias plot (mean concentration plotted against difference between concentrations) indicated good agreement between methods when ACTH concentrations were < 100 pg/mL; when concentrations were > 100 pg/mL, the values obtained by use of the immunoradiometric assay described here yielded values that were 10% to 25% lower than values for the previously validated assay. The reference range for plasma endogenous ACTH concentrations was calculated as 10 to 80 pg/mL for both assays. A total of 29 samples from ill dogs were assayed to determine plasma endogenous ACTH concentrations; 2 samples were inadvertently misplaced during the study. In ill dogs, all samples were assayed for endogenous ACTH concentrations in duplicate in a single batch. For healthy dogs, plasma samples for endogenous ACTH concentrations were assayed in duplicate in multiple batches, but samples from each dog were assayed in a single batch when possible.

Assessment of clinical variables—Additional data for each ill dog (including duration in the hospital and ICU; serum sodium and potassium concentrations, sodium-potassium ratio, and use of vasopressors; and detection of hypoxemia, cardiac arrhythmias, and hypotension) were recorded at the time of ACTH-stimulation testing. Screening for hypoxemia was accomplished via pulse oximetry or arterial blood gas analysis; however, all patients suspected of being hypoxemic by use of pulse oximetry were confirmed by arterial blood gas analysis. A patient was considered to be hypoxemic when PaO_2 was < 80 mm Hg while breathing room air.²⁵ Cardiac arrhythmias were determined by evaluation of a lead II ECG. Blood pressure determination was performed by indirect Doppler or direct arterial blood pressure measurement. Systolic blood pressure < 80 mm Hg or mean arterial blood pressure < 60 mm Hg by either method of measurement was considered hypotensive.²⁶ All ill dogs were treated identically with regard to the initial treatment for hypotension (administration of crystalloids, colloids, or blood products). When blood pressure did not increase after adequate fluid administration, vasopressors were started. Survival and mortality data (ie, survival until discharge from hospital, death during hospitalization, or euthanasia during hospitalization) were also recorded for each patient.

Statistical analysis—The Shapiro-Wilk statistical test for normality was used to determine that data were normally distributed; therefore, parametric statistical analyses were performed. Results are reported as mean \pm SEM. The Student *t* test was used to compare age, body weight, and sex between critically ill and healthy dogs. A 1-way ANOVA was used to compare results for ACTH-stimulation testing between dogs tested during or after surgery and those treated medically or that were tested

before surgery as well as to compare data among groups of critically ill dogs (ie, septic, severe trauma, and GDV). The Pearson correlation coefficient was used to assess the correlation between baseline plasma endogenous ACTH and serum cortisol concentrations. The χ^2 test of independence and 1-way ANOVA were used to compare differences in morbidity rates among groups of dogs. Groups that were compared included ill dogs that had biochemical abnormalities indicative of insufficiency of the adrenal gland or pituitary gland with those that did not have such abnormalities, dogs that had no apparent response to ACTH stimulation (ie, dogs with high baseline serum cortisol concentrations and Δ -cortisol ≤ 1 nmol/L) with dogs that had a normal response to ACTH stimulation, and dogs with Δ -cortisol ≤ 83 nmol/L with those that had Δ -cortisol > 83 nmol/L. Morbidity factors that were analyzed included duration of hospitalization; duration of ICU stay; use of vasopressors; and detection of hypoxemia, cardiac arrhythmias, and hypotension. When a response variable was quantitative (ie, number of days in hospital or number of days in ICU), a 1-way ANOVA was used to assess whether the means for the outcome groups differed. For qualitative response variables (eg, use of vasopressors or detection of hypoxemia), a χ^2 test of independence was used. Significant results were reported as the relative risk, which predicted the rate of event incidence in 1 population in comparison with the rate in another population (with 95% confidence intervals). The χ^2 test of independence was also used to compare differences in outcome (ie, survival until discharge vs death during hospitalization) among groups of dogs. The same groups that were compared for the morbidity factor analyses were also used for the outcome analyses. However, dogs that were euthanatized were censored from the outcome analyses because of the inability to determine whether the euthanatized dogs would have died from their illness or would have recovered after appropriate treatment. Statistical analyses were performed by use of a statistical software package.¹ For all analyses, values of $P \leq 0.05$ were considered significant.

Results

Critically ill dogs ranged from 1.4 to 12.5 years of age (mean \pm SEM, 5.9 ± 0.6 years) and weighed 2.0 to 53.3 kg (4.4 to 117.3 lb), with a mean of 31.8 ± 3.6 kg (70.0 ± 7.9 lb). There were 8 neutered females, 5 sexually intact females, 11 neutered males, and 7 sexually intact male dogs. The most common breeds represented included 7 mixed-breed dogs, 3 Labrador Retrievers, 2 Saint Bernards, and 2 Australian Shepherds. Healthy dogs ranged from 1 to 8 years of age (mean, 4.3 ± 0.9 years) and weighed 17.6 to 42.3 kg (38.7 to 93.1 lb), with a mean of 28.2 ± 2.5 kg (62.0 ± 5.5 lb). There were 6 female and 4 male dogs; all were spayed or neutered. Breeds represented included 5 mixed-breed dogs, 2 Labrador Retrievers, 1 Plott Hound, 1 Australian Cattle Dog, and 1 Dalmatian. Age and body weight did not differ significantly between critically ill and healthy dogs; however, there were proportionally more males in the critically ill dogs than in the healthy dogs.

No correlation was found between mean \pm SEM baseline plasma endogenous ACTH (63 ± 18 pg/mL)

and mean baseline serum cortisol (217 ± 19 nmol/L) concentrations in the overall population of critically ill dogs. For comparison purposes, baseline plasma endogenous ACTH and serum cortisol concentrations from the 10 healthy dogs were analyzed; results were within the reference ranges for both hormonal variables. The correlation between mean baseline plasma endogenous ACTH (32 ± 3 pg/mL) and mean baseline serum cortisol (79 ± 9 nmol/L) concentrations in healthy dogs had a significant positive correlation ($r = 0.573$; $P < 0.001$).

Of the 31 critically ill dogs, 13 (42%) were septic, 10 (32%) had severe traumatic injuries, and 8 (26%) had GDV. Nine of 13 (69%) septic dogs had peritonitis secondary to rupture of an abdominal abscess (ie, hepatic or prostatic abscess or pyometra) or rupture of an abdominal viscus (ie, intestinal tract or gall bladder), 3 of 13 (23%) had severe cellulitis, and 1 of 13 (8%) had aspiration pneumonia. Microbial culture revealed several organisms, including *Escherichia coli*, *Pasteurella multocida*, *Streptococcus canis*, *Clostridium perfringens*, *Enterococcus* spp, *Proteus* spp, and *Cedecea davisae*. The most commonly cultured organism was *E coli* (6/13 [46%]). More than 1 organism was cultured from 6 of 13 (46%) septic dogs. Eight of 10 trauma dogs were involved in motor vehicle accidents, and 2 were attacked by other dogs.

Duration of hospitalization for the 31 critically ill dogs ranged from 1 to 14 days (mean \pm SEM, 4.7 ± 0.7 days), and duration of ICU stay ranged from 1 to 14 days (mean, 3.9 ± 0.7 days). Serum sodium concentrations for the study population ranged from 136.9 to 169 mEq/L (mean, 149.3 ± 1.4 mEq/L; reference range, 145 to 157 mEq/L), and serum potassium concentrations ranged from 2.8 to 4.9 mEq/L (mean, 3.9 ± 0.1 mEq/L; reference range, 4.4 to 5.3 mEq/L). Sodium-to-potassium ratios ranged from 30:1 to 57:1 (mean, 39.1 ± 1.1 ; reference range, $> 27:1$). Eight of 31 (26%) dogs were hypoxemic at the time of ACTH-stimulation testing, with P_{aO_2} values while breathing room air ranging from 40 to 78.5 mm Hg (mean 65.1 ± 9.0 mm Hg; reference range, > 80 mm Hg). Twelve of 31 (39%) dogs had cardiac arrhythmias at the time of ACTH-stimulation testing; all had ventricular premature contractions or ventricular tachycardia. Twelve of 31 (39%) dogs were hypotensive at the time of ACTH-stimulation testing, with systolic blood pressure ranging from 44 to 78 mm Hg (mean, 62.6 ± 6.9 mm Hg; reference range, > 80 mm Hg). Six of 12 hypotensive dogs were receiving vasopressors at the time of ACTH-stimulation testing; all 6 were septic dogs. The remaining 6 hypotensive dogs were receiving treatments consisting of crystalloids, colloids, blood products, or a combination thereof.

The ACTH-stimulation testing was performed in 12 of 31 (39%) dogs during or after surgery, whereas 19 of 31 (61%) dogs were tested before surgery or received medical treatment only. Mean baseline plasma endogenous ACTH, mean baseline serum cortisol, and mean ACTH-stimulated serum cortisol concentrations and mean Δ -cortisol were compared between dogs tested during and after surgery and those tested before surgery or treated medically; no significant differences were detected. Therefore, data for all critically ill dogs were combined for further statistical analysis. When

comparing septic dogs, dogs with severe trauma, and dogs with GDV, we did not detect significant differences among groups with respect to mean baseline plasma endogenous ACTH, baseline serum cortisol, and ACTH-stimulated serum cortisol concentrations or Δ -cortisol (Table 1).

Overall, 17 of 31 (55%) dogs (7 dogs with sepsis, 6 dogs with severe trauma, and 4 dogs with GDV) had at least 1 biochemical abnormality consistent with adrenal gland or pituitary gland insufficiency. None of the critically ill dogs in the study had a baseline serum cortisol concentration less than the reference range (Table 2). Of the dogs with sepsis, 5 had an ACTH-stimulated serum cortisol concentration less than the reference range; 1 of those 5 dogs also had a plasma endogenous ACTH concentration less than the reference range. Two septic dogs had no apparent response to ACTH stimulation (baseline serum cortisol concentrations of 299 and 263 nmol/L and ACTH-stimulated serum cortisol concentrations of 298 and 259 nmol/L, respectively). Of the dogs with severe trauma, 4 had no apparent response to ACTH stimulation (baseline serum cortisol concentrations of 299, 298, 322, and 272 nmol/L and ACTH-stimulated serum cortisol concentrations of 300, 281, 293, and 257 nmol/L, respectively) and 3 had a plasma endogenous ACTH concentration less than the reference range. One dog that did not have an apparent response to ACTH stimulation (baseline and ACTH-stimulated serum cortisol concentration of 322 and 293 nmol/L, respectively) also had a low plasma endogenous ACTH concentration (9.1 pg/mL). Of the dogs with GDV, 2 had ACTH-stimulated serum cortisol concentrations less than the reference range, 2 had a plasma endogenous ACTH concentration less

than the reference range, and 1 did not have an apparent response to ACTH stimulation (baseline serum cortisol concentration of 289 nmol/L and ACTH-stimulated serum cortisol concentration of 264 nmol/L). One dog that had a low ACTH-stimulated serum cortisol concentration (202 nmol/L) also had a low plasma endogenous ACTH concentration (5.8 pg/mL).

Conversely, 19 of 31 (61%) dogs (6 dogs with sepsis, 8 dogs with severe trauma, and 5 dogs with GDV) had at least 1 biochemical abnormality consistent with pituitary gland or adrenal gland overactivity. Nineteen dogs had a baseline serum cortisol concentration greater than the reference range (Table 2). However, this included the 7 dogs that had no apparent response to ACTH stimulation, with Δ -cortisol \leq 1 nmol/L. Only 1 (3%) dog (a dog with sepsis) had an ACTH-stimulated serum cortisol concentration greater than the reference range. Five of 29 (17%) dogs had a plasma endogenous ACTH concentration greater than the reference range.

None of the 17 dogs with biochemical abnormalities suggestive of adrenal gland or pituitary gland insufficiency had classic electrolyte changes (ie, hyponatremia and hyperkalemia) that are typically evident with complete adrenocortical insufficiency. Mean \pm SEM serum sodium concentration in these dogs was 149.1 ± 1.8 mEq/L (range, 140 to 169 mEq/L). Mean serum potassium concentration was 4.0 ± 0.1 mEq/L (range, 3.2 to 4.9 mEq/L). Mean sodium-to-potassium ratio was $38:1 \pm 1:1$ (range, 30:1 to 53:1).

Mean \pm SEM duration of hospitalization and ICU stay for the 17 dogs with adrenal gland or pituitary gland insufficiency was 4.4 ± 1.0 days and 3.7 ± 1.0 days, respectively. For the remaining 14 dogs, the mean

Table 1—Mean \pm SEM (range) values for plasma endogenous ACTH, baseline serum cortisol, and ACTH-stimulated serum cortisol concentrations and Δ -cortisol in 31 dogs with acute critical illness.

Type of illness	No. of dogs	Endogenous ACTH concentration (pg/mL)	Baseline cortisol concentration (nmol/L)	ACTH-stimulated cortisol concentration (nmol/L)	Δ -Cortisol (nmol/L)
Sepsis	13	71.9 \pm 30.3 (8.8 to 421.8)	179.8 \pm 28.7 (44 to 345)	280.6 \pm 44.0 (54 to 858)	100.8 \pm 32.7 (–4 to 513)
Trauma	10	80.6 \pm 31.8 (2.6 to 316.5)	254.9 \pm 32.8 (91 to 418)	360.7 \pm 50.2 (257 to 551)	105.8 \pm 37.3 (–29 to 271)
GDV	8	29.9 \pm 35.6 (5.5 to 69.8)	231.6 \pm 36.6 (114 to 476)	341.6 \pm 56.1 (202 to 542)	110 \pm 41.7 (–25 to 226)

Table 2—Number, percentage, and range values for 31 dogs with acute critical illness that had concentrations of serum cortisol or plasma endogenous ACTH outside the respective reference ranges.*

Type of illness	Variable	Less than reference range			Greater than reference range		
		Baseline cortisol concentration	ACTH-stimulated cortisol concentration	Baseline endogenous ACTH concentration	Baseline cortisol concentration	ACTH-stimulated cortisol concentration	Baseline endogenous ACTH concentration
Sepsis (n = 13)	No. (%)	0 (0)	5 (16)	1 (3)	6 (19)	1 (3)	2 (7)
	Range	—	54 to 170 nmol/L	8.8 pg/mL	215 to 345 nmol/L	858 nmol/L	89.9 to 421.8 pg/mL
Trauma (n = 10)	No. (%)	0 (0)	0 (0)	3 (10)	8 (26)	0 (0)	3 (10)
	Range	—	—	2.6 to 9.1 pg/mL	170 to 418 nmol/L	—	118.9 to 316.5 pg/mL
GDV (n = 8)	No. (%)	0 (0)	2 (7)	2 (7)	5 (16)	0 (0)	0 (0)
	Range	—	202 to 219 nmol/L	5.5 to 5.8 pg/mL	177 to 476 nmol/L	—	—
Total (n = 31)	No. (%)	0 (0)	7 (23)	6 (21)	19 (61)	1 (3)	5 (17)
	Range	—	54 to 219 nmol/L	2.6 to 9.1 pg/mL	170 to 476 nmol/L	858 nmol/L	89.9 to 421.8 pg/mL

*Reference range for baseline serum cortisol concentration is 10 to 160 nmol/L, reference range for ACTH-stimulated serum cortisol concentration is 220 to 560 nmol/L, and reference range for baseline plasma endogenous ACTH concentration is 10 to 80 pg/mL.
— = Not applicable.

duration of hospitalization and ICU stay was 5.1 ± 0.9 days and 4.3 ± 0.9 days, respectively. Four of 17 (24%) dogs with adrenal gland or pituitary gland insufficiency were hypoxemic, 7 (41%) had cardiac arrhythmias, and 6 (35%) were hypotensive at the time of ACTH-stimulation testing. Similarly, 4 of the remaining 14 (29%) dogs without adrenal gland or pituitary gland insufficiency were hypoxemic, 5 (36%) had cardiac arrhythmias, and 6 (43%) were hypotensive at the time of ACTH-stimulation testing. Vasopressors were used in 5 of 17 (29%) dogs with adrenal gland or pituitary gland insufficiency, whereas only 1 of the remaining 14 dogs received vasopressors. When results for morbidity factors (ie, duration of hospitalization; duration of ICU stay; use of vasopressors; and detection of hypoxemia, cardiac arrhythmias, or hypotension) for those dogs with a biochemical abnormality consistent with adrenal gland or pituitary gland insufficiency were compared with those for dogs that did not have a similar biochemical abnormality, no significant differences were detected. Similarly, when results for morbidity factors for dogs that had no apparent response to ACTH stimulation (dogs with high baseline serum cortisol concentrations and Δ -cortisol ≤ 1 nmol/L) were compared with those for dogs with a normal response to ACTH stimulation, no significant differences were detected. However, when results for morbidity factors for dogs with Δ -cortisol ≤ 83 nmol/L were compared with those for dogs with Δ -cortisol > 83 nmol/L, dogs with Δ -cortisol ≤ 83 nmol/L were 5.7 times as likely to be receiving vasopressors as were dogs with Δ -cortisol > 83 nmol/L (relative risk, 5.7; 95% confidence interval, 3.7 to 7.7; $P = 0.036$). Five of 6 dogs that were receiving vasopressors had Δ -cortisol ≤ 83 nmol/L.

Three (10%), 7 (23%), and 21 (68%) critically ill dogs in the study died, were euthanatized, or survived until discharge from the hospital, respectively. Of the 3 dogs that died, 2 had severe trauma and 1 had sepsis. Of the 7 dogs that were euthanatized, 5 had sepsis, 1 had severe trauma, and 1 had GDV. Of the 21 dogs that survived until discharge from the hospital, 7 had sepsis, 7 had severe trauma, and 7 had GDV. However, 1 dog with severe trauma that survived until discharge from the hospital was readmitted 2 days later and died 6 hours after readmission as a result of postoperative complications including sepsis, disseminated intravascular coagulation, and pulmonary thromboembolism. When outcome for dogs with a biochemical abnormality consistent with adrenal gland or pituitary gland insufficiency was compared with outcome for dogs without such abnormalities, no significant difference was detected. When dogs with no apparent response to ACTH stimulation were compared with those that had a normal response to ACTH stimulation, outcome also did not differ significantly ($P = 0.053$) between these 2 groups. Similarly, when dogs with Δ -cortisol ≤ 83 nmol/L were compared with those that had Δ -cortisol > 83 nmol/L, outcome did not differ significantly ($P = 0.055$) between the 2 groups.

Discussion

Biochemical abnormalities consistent with dysfunction of the HPA axis were common in the acutely critically ill dogs of the study reported here. Although

the relative frequency of each type of abnormality was low, 17 (55%) dogs had at least 1 biochemical abnormality suggestive of adrenal gland or pituitary gland insufficiency. These abnormalities were detected in all 3 groups of critically ill dogs. Septic dogs and dogs with GDV were identified that had an ACTH-stimulated serum cortisol concentration less than the reference range, whereas dogs from all 3 groups were identified that had no apparent response to ACTH stimulation or that had plasma endogenous ACTH concentrations less than the reference range.

The results of our study and those of another study¹⁵ suggest that there may be impaired adrenal gland or pituitary gland function in some critically ill dogs. In the other study,¹⁵ investigators assessed pituitary-adrenal function in 33 septic dogs admitted to an ICU; baseline plasma endogenous ACTH and ACTH-stimulated serum cortisol concentrations less than the reference range were detected, and Δ -cortisol ≤ 3 μ g/dL (83 nmol/L) were associated with systemic hypotension and a decrease in survival. Accordingly, we elected to examine this cut-off point for Δ -cortisol in our critically ill dogs. Our results were similar, in that dogs with Δ -cortisol ≤ 83 nmol/L were more likely to be receiving vasopressors and had a slight decrease in survival, although the decrease in survival was not significant ($P = 0.055$).

Other investigators have evaluated pituitary-adrenal function in critically ill dogs²⁷ and in dogs with severe illness attributable to nonadrenal gland disease²⁸ and did not detect adrenal gland insufficiency because none of the dogs had an ACTH-stimulated serum or plasma cortisol concentration less than the reference range. No data were available regarding Δ -cortisol values in either of those studies. The difference between studies could be attributable to various factors. First, the duration of illness differed. Only 40% of the dogs enrolled by investigators in one of those studies²⁷ were acutely ill, and dogs in the other study²⁸ typically had chronic diseases, with the duration of morbidity ranging from 1 week to 1 year (mean, 5.8 ± 1.4 weeks). Second, both studies encompassed a general population of ICU dogs with an array of diseases. Only 3 of the 20 critically ill dogs in 1 study²⁷ had sepsis, and no other dogs in either study^{27,28} had severe trauma or GDV.

Despite these differences, results of the study reported here and those of other studies^{15,27,28} have revealed that critically ill dogs typically have baseline serum or plasma cortisol concentrations that are within or greater than the reference range. None of the studies detected low baseline serum or plasma cortisol concentrations. Thus, given that dogs can still have no or a sub-optimal response to ACTH stimulation, measurement of baseline serum or plasma cortisol concentrations is inadequate to diagnose adrenal gland insufficiency in critically ill dogs, and assessment of the patient's response to ACTH administration is required.

Our study, in addition to those of other investigators,^{15,27} revealed that some critically ill dogs had a decrease in plasma endogenous ACTH concentrations. Other investigators did not measure plasma endogenous ACTH concentrations as part of their study.²⁸ Detecting baseline serum or plasma cortisol concentrations within or greater than the reference range in combination with

a decrease in baseline plasma endogenous ACTH concentrations may signal loss of correlation between basal endogenous ACTH and cortisol concentrations, which has been reported in critically ill human patients.^{3,4,29,30} One possible explanation is that cortisol clearance is decreased during periods of stress or illness, which leads to an increase in serum cortisol concentrations and enhanced negative feedback to the pituitary gland.³¹ In addition, ACTH may induce upregulation of its own receptors during periods of stress or illness, thus enhancing the cortisol response to ACTH.^{32,33} Finally, factors other than ACTH, especially interleukin-6 and tumor necrosis factor- α , can directly stimulate glucocorticoid synthesis by the adrenal gland and bypass the HPA axis.^{32,33} Alternatively, because plasma endogenous ACTH concentrations can vary greatly over time as a result of the short half-life in circulation and because clinically normal dogs can have periods of dissociation between ACTH and cortisol concentrations,³⁴ random sampling may not reflect the true reserve or response of the pituitary gland. However, because a correlation was detected in our population of healthy dogs, random sampling may not be the issue.

The pathophysiologic mechanisms for the pituitary-adrenal alterations in our critically ill dogs were unclear. For dogs with adrenal gland insufficiency, disturbance of the pituitary-adrenal axis may have been initiated at the pituitary gland. In the face of adrenal gland insufficiency, negative feedback on the pituitary gland is lacking and plasma endogenous ACTH concentrations should be increased. In other words, an endogenous ACTH concentration within the reference range in a patient with a diminished response to ACTH stimulation is suggestive of pituitary gland insufficiency. In 6 of 7 dogs with an ACTH-stimulated serum cortisol concentration less than the reference range, plasma endogenous ACTH concentrations were less than (2 dogs) or within (4 dogs) the reference range. The 1 remaining dog with an ACTH-stimulated serum cortisol concentration less than the reference range was one of the dogs in which the sample for measurement of endogenous ACTH concentration was inadvertently misplaced. The cause of the pituitary gland disturbance is unknown. In critically ill humans, sepsis and systemic inflammatory response syndrome are the most common causes of RAI,⁹ likely attributable in most cases to the release of cytokines and other inflammatory mediators that suppress the release of CRH and ACTH.^{6,35} Interleukin-6 appears to be one of the most potent stimuli of ACTH secretion in humans.^{6,36} In septic patients, a blunted interleukin-6 response may result in understimulation of the HPA axis, which contributes to RAI.³⁷ In addition, tumor necrosis factor- α impairs CRH-stimulated secretion of ACTH.³⁸

All 7 dogs with no apparent response to ACTH stimulation had high baseline serum cortisol concentrations. The lack of response suggests that these dogs already had maximal endogenous adrenocortical stimulation and reflected a depletion of the reserve capacity for adrenocortical secretion. These 7 dogs are comparable to humans classified as having RAI (baseline serum cortisol concentration within or greater than the reference range and a poor response to ACTH stimula-

tion [ie, low Δ -cortisol]). Much debate exists regarding the appropriate method for diagnosing RAI in humans. However, one of the most accepted methods is to determine Δ -cortisol after a standard 250- μ g ACTH-stimulation test. This method is largely based on results of a study¹¹ that indicated an association between Δ -cortisol ≤ 9 μ g/dL and a decrease in survival as well as an association between glucocorticoid treatment and an improved outcome for humans with Δ -cortisol ≤ 9 μ g/dL. Although the clinical implications of RAI in veterinary medicine are not clear, our results raise the question of whether the administration of physiologic dosages of glucocorticoids will benefit dogs with acute critical illness and similar results for ACTH-stimulation tests. It has been hypothesized that human patients who cannot increase cortisol secretion after ACTH administration constitute a subgroup of patients who require glucocorticoid supplementation despite high baseline concentrations.³⁹ Moreover, the poor response to ACTH stimulation in these 7 dogs may signal adrenocortical exhaustion as well as represent a prognostic indicator for poor patient outcome. Human patients in septic shock with high baseline cortisol concentrations combined with a weak cortisol response to ACTH had an increased risk of death, with the 28-day mortality rate as high as 82%. Mortality risk diminished and, correspondingly, 28-day survival improved in patients with lower baseline cortisol concentrations and appropriate cortisol responses.⁴⁰ Even though outcome analysis for the comparison between dogs with no apparent response to ACTH stimulation and those with a normal response did not reveal a significant difference in mortality rate, it is interesting that all 3 dogs that died prior to discharge from the hospital had no apparent response to ACTH stimulation.

None of the dogs with pituitary gland or adrenal gland insufficiency had the classic electrolyte changes (ie, hyponatremia and hyperkalemia) evident with complete adrenocortical insufficiency. Even though serum aldosterone concentrations were not measured in this study, the lack of electrolyte perturbances suggests that mineralocorticoid concentrations were sufficient. The role of concurrent aldosterone insufficiency as a component of RAI is unclear in critically ill human patients; however, concurrent hyponatremia and hyperkalemia is not a common clinical manifestation of RAI.^{35,41-43}

Studies in dogs with nonadrenal gland illnesses,^{28,44} critical illnesses,²⁷ and neoplasia¹⁴ revealed that 9% to 36% had an ACTH-stimulated serum cortisol concentration greater than the reference range. Therefore, it is surprising that only 1 (3%) dog in the study reported here had an exaggerated response to ACTH. A possible explanation is that the dogs in our study were acutely ill, and the HPA axis may not have had time to fully adapt. The acute and chronic responses of the HPA axis to illness differ, and the acute response may not always be predictive of the chronic response.⁹ Exaggerated cortisol responses may be more consistent with sustained activation of the HPA axis, such as those in dogs with chronic illness.^{14,27,28,44}

The study reported here had a few limitations. First, group sizes were relatively small. The lack of statistical differences among the 3 groups of dogs with respect

to mean baseline serum cortisol, ACTH-stimulated serum cortisol, and plasma endogenous ACTH concentrations and Δ -cortisol could have been attributable to small sample size and should be interpreted cautiously. This is also the likely scenario for the failure to detect significant differences in the outcome analysis when comparing dogs with no apparent response to ACTH stimulation with dogs that had a normal response and when comparing dogs with Δ -cortisol ≤ 83 nmol/L with those that had Δ -cortisol > 83 nmol/L. Second, artifact could have been introduced by failure to identify dogs that may have received glucocorticoids, ketoconazole, or etomidate, despite careful questioning of owners and referring veterinarians. In many cases, referring veterinarians were contacted to confirm the medical history. Additionally, the decision to limit the exclusion criteria for glucocorticoid use to only the preceding 4 weeks may not have been sufficient for all dogs. Third, even though no significant differences were detected when mean baseline plasma endogenous ACTH, baseline serum cortisol and ACTH-stimulated serum cortisol concentrations, and Δ -cortisol were compared between patients tested during and after surgery with those treated medically or that had their pituitary-adrenal axis assessed before surgery, merging the 2 groups of dogs may have been suboptimal. To our knowledge, the effect of anesthesia on ACTH-stimulated cortisol concentration in dogs has not been evaluated. Fourth, total serum cortisol concentrations may not accurately represent the biological activity of cortisol during acute critical illness in dogs. Assays for total serum cortisol measure the total hormone concentration (ie, serum free cortisol plus the protein-bound fraction). Because serum free cortisol is believed to be responsible for the physiologic function of the hormone,^{45,46} serum free cortisol concentrations may be a more precise predictor of adrenal gland function in critically ill patients.⁴⁵ Alterations in protein binding (which could be evident in critically ill patients) may affect free cortisol concentration, and serum total cortisol concentration may not be an accurate reflection of free cortisol concentration.^{39,46} Unfortunately, at the time the study was performed, an assay for serum free cortisol concentrations in dogs was not available.

In the study reported here, there was evidence of adrenal gland and pituitary gland insufficiency in the dogs with acute critical illnesses, although an exaggerated response to ACTH administration was uncommon. There was a loss of correlation between baseline plasma endogenous ACTH and serum cortisol concentrations, which has been reported in other studies of critically ill human and veterinary patients. In addition, no differences were detected among dogs with sepsis, severe trauma, or GDV with respect to mean baseline serum cortisol, ACTH-stimulated serum cortisol and plasma endogenous ACTH concentrations, and Δ -cortisol. The exact pathophysiologic mechanisms, clinical importance, and therapeutic implications remain to be elucidated. It is unclear whether some critically ill dogs may benefit from physiologic doses of glucocorticoids. However, acutely ill dogs with Δ -cortisol ≤ 83 nmol/L appeared to be more likely to require vasopressors, and dogs with no apparent response to ACTH stimulation or

with Δ -cortisol ≤ 83 nmol/L had a slight but not significant increase in fatalities. Additional studies examining abnormalities of the HPA axis in critically ill dogs are warranted. Although CRH-stimulation testing is currently used in humans to distinguish between ACTH and CRH deficiency,⁹ to our knowledge, its use has not been described in dogs. Use of CRH-stimulation testing may help elucidate the pathogenesis of dysfunction of the HPA axis in critically ill dogs and perhaps allow for the detection of dogs that may benefit from glucocorticoid supplementation.

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