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BLOOD GAS AND CATECHOLAMINE LEVELS IN CAPTURE STRESSED DESERT BIGHORN SHEEP

Richard W. Martucci,[1] David A. Jessup,[2,3] Gerald A. Gronert,[1] John A. Reitan,[1] and William E. Clark[3]

[1] Department of Anesthesiology, School of Medicine, University of California, Davis, California 95616, USA
[2] Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616, USA
[3] California Department of Fish and Game, 1701 Nimbus Road, Rancho Cordova, California 95670, USA

ABSTRACT: Forty-seven bighorn sheep (*Ovis canadensis nelsoni*) were captured within a 3-day period in December, 1989 as part of a California Department of Fish and Game effort to repopulate historic ranges in California. They were captured on the Mojave Desert in the Kelso Mountains near Old Dad Peak, San Bernardino County, California. Venous blood gases measured at the site of capture demonstrated a severe metabolic acidosis (base deficit, 23 mEq/liter), with no evidence of respiratory acidosis. There were moderately elevated plasma epinephrine (1.25 ng/ml), norepinephrine (2.60 ng/ml), and dopamine (114 pg/ml) levels. These data appear to reflect animals that have been moderately stressed. These acid-base-catecholamine values differ from values in resting domestic sheep, and are similar to those reported in greyhounds after brief strenuous exercise.

Key words: Bighorn sheep, *Ovis canadensis*, wildlife capture, capture stress, acid-base balance, catecholamine, epinephrine, norepinephrine, dopamine.

INTRODUCTION

Over the past 10 yr, the California Department of Fish and Game has actively reintroduced bighorn sheep into historic habitats, an endeavor involving at least 18 capture and translocation efforts. The effects of capture, handling, transport and release are stressful to wild animals. Mortality rates have varied from <2% with net-gun capture to 4% with drive-net and drop-net capture and higher for darting and drive-trapping. Deaths are due in part to direct trauma, exhausting exercise in an otherwise normal animal, or capture myopathy (CM). These possibilities prompt a brief consideration of capture stress (CS) and CM.

Stress is a situation that is associated with fluctuations in metabolism, cardiovascular activity, sympathetic nervous responses, and various hormones and electrolytes (Weissman, 1990). Factors that help to delineate stress include O_2 consumption, CO_2 production, indirect estimates of these via blood gases and ventilation, catecholamines, enzymes such as creatine kinase (CK), potassium, lactate, acid-base status, adrenal steroids, and glucose. Most fit subjects tolerate modest degrees of stress and recover easily from moderate alterations in the above parameters. The conditioned individual tolerates greater degrees of stress without undue adverse responses. However, every animal has a limit. Capture myopathy resembles either rigorous exercise to the point of exhaustion (march myogloginuria in man or Monday Morning Disease in horses), or an exaggerated response to stress, as with malignant hyperthermia (MH) which is a genetic disorder. We investigated whether the stress responses of bighorn sheep were exaggerated, as in MH and if captive stress levels bordered on those which could result in CM. Thus, we examined external jugular venous blood gas values at the time of capture, with the intention that compromised animals might be immediately treated to ensure survival. We also assessed acid-base balance, levels of stress related hormones, glucose, and CK values. Venous blood gases provided a measure of blood flow and O_2 delivery, ventilation and clearance of CO_2, and acid base balance. Catecholamines (epinephrine, norepinephrine, dopamine) served as an indirect indicator of sympathetic stimulation, CK as an indicator of stability of muscle permeability, and glucose values as the end product of the effects of catecholamines, glucocorticoids, and tissue utilization and excretion.

MATERIAL AND METHODS

Forty-seven bighorn sheep were captured in December 1989 near Old Dad Peak in the Kelso Mountains, California (34°12′N, 116°58′W). Capture was in the morning and early afternoon at an ambient temperature of 40 to 50 F (4.4 to 10 C). Thirty-nine females and nine males ranging in ages from 0.5- to 11-yr-old were captured using the helicopter net-gun method (Jessup et al., 1984). In this procedure an individual animal is located, maneuvered into position from the air, and entrapped by a 5 × 5 m nylon net shot from the net-gun (Coda Enterprises, Inc., Mesa, Arizona 85201, USA). The helicopter lands and the animal is shackled and blindfolded as soon as possible. Initial rectal temperature is measured and recorded. Water soaking was used to cool down animals overheated by pursuit. Care was taken to minimize chase times to decrease stress and overheating. In some situations animals were allowed to rest before final chase.

Blindfolded animals were transported to base camp in plastic mesh bags suspended by cable below the helicopter. Water soaked animals cooled during air transport. At base camp a quick but thorough physical examination was performed, temperature measured again, prophylactic medications (penicillin, vitamin E and selenium, 7-way clostridial toxoid) and emergency or other treatments given if needed, identity tags and radio tracking collars placed, and biological data (various weights and measures) obtained. Heparinized jugular venous blood samples were collected during physical restraint. In the afternoon, work was done on a wet carpet under a shade cloth at base camp. No immobilizing drugs were used. Every effort was made to follow a consistent gentle procedure in the capture and handling process. Age of bighorn sheep was determined using counts of horn rings and tooth eruption (Geist, 1966).

Venous blood pH and blood gases, oxygen tension (PO_2) and carbon dioxide tension (PCo_2), were determined directly on a generator powered Corning Model 170 auto-calibrating blood gas machine (Ciba Corning Diagnostics Corp., Medfield, Massachusetts 02052, USA) located in an insulated van at the base camp site. External jugular (EJ) PO_2 values reflect head and brain O_2 demands and the degree to which blood flow meets these demands. EJ PCo_2 values indicate the degree of pulmonary ventilation relative to CO_2 production; human venous PCo_2 is greater than arterial PCo_2 by about 0.8 KPa (6 mm Hg) when blood flow is adequate (Adrogué et al., 1989). pH and PCo_2 together determine the degree of respiratory and metabolic acidosis.

Within 4 min after blood samples were drawn, results were provided to the processing team as a potential aid in determining treatment. Base excess values were calculated using a hemoglobin (Hb) concentration of 16.6 g/dl, based upon mean values of free-ranging bighorn sheep (*Ovis canadensis*) reported by Kock et al. (1987b) from previous captures (n = 465).

For catecholamine analysis, 6 to 10 ml of venous blood was collected in heparinized vacuum tubes (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey 07070, USA). Five ml was immediately transferred to a prechilled 5 ml vacuum tube containing ethyleneglycol-bis-(B-aminoethylether) N, N, N, N,-tetra-acetic-acid (EDTA) and reduced glutathione (CAT-A-KIT blood collection tubes, Amersham Corp., Arlington Heights, Illinois 60005, USA). Samples were centrifuged at 600 g for 15 min in a generator powered centrifuge. The centrifuge was not refrigerated, but the removable metal centrifuge holders were kept in an ice chest between uses to minimize warming of samples during centrifugation. Plasma was stored in a dry ice cooler for transport to the laboratory. At the laboratory, samples were stored at −70 C. Time between collection in the field to storage at −70 C ranged from 24 to 72 hr. Plasma catecholamines (norepinephrine, epinephrine, and dopamine) were analyzed by high pressure liquid chromatography (HPLC) with electrochemical detection on a RMC 100 C18 column (Waters Chromatography Division, Milford, Massachusetts 01757, USA). The method (Weicker et al., 1984) has a calculated sensity of 100 pg/ml. Standard methods were used for analysis of CK and blood glucose (NaF preservative) Kock et al., 1987a). Data are expressed as mean ± SE.

RESULTS

Data from 39 female and eight male bighorn sheep are shown in Table 1. Mean age was 4.4 ± 0.5 yrs, range 0.5 to 11 yr. There were three mature males between 4 and 9 yr of age. The group included one 11-yr-old female and three lambs. Mean weight was 45 ± 1 Kg, range 30 to 71 Kg.

Bighorn sheep venous acid-base data (Table 1) demonstrate metabolic acidosis. Venous PCo_2 values are usually 0.8 KPa (6 mm Hg) greater than those in arterial blood in humans, dogs, and swine (Adrogué et al., 1989; Gronert and Theye, 1976; Mich-

TABLE 1. Blood gas, glucose, creatine kinase (CK), body temperature, and catecholamine values of recently captured desert bighorn sheep.

	Number	Mean	Standard error	Range
External jugular venous blood gas values				
pH	45	7.02	0.02	6.69–7.39
PCo_2 (kPa) (mm Hg)	45	3.7 (28)	1	2.5–5.9 (19–44)
PO_2 (kPa) (mm Hg)	45	8.0 (60)	2	4.4–10.7 (33–80)
base excess (mEq/l)	45	−23	1	−34– − 4
Catecholamines				
Norepinephrine (ng/ml)	45	2.60	0.17	0.75–5.37
Epinephrine (ng/ml)	45	1.25	0.12	0.25–3.48
Dopamine (pg/ml)	42	114	13	20–494
Other				
Creatine kinase (U/l)	17	637	110	217–1,729
Glucose (mg/dl)	17	156	9	88–248
Rectal temperature (C) at capture	43	40.8	0.2	38.8–42.9
Rectal temperature (C) when sampled	47	40.6	0.1	37.8–42.4

enfelder and Theye, 1968). Thus, in this group, with a mean venous PCo_2 of 3.7 KPa (28 mm Hg), PCo_2 values in arterial blood were likely near the lower range of normal. Jugular venous PO_2 values were greater than baseline brain venous PO_2 values (Michenfelder and Theye, 1968). Brain venous PO_2 values under anesthesia may influence jugular venous values more so than scalp or head muscles because these muscles and tissues are not active. Catecholamine levels (Table 1) were greater in net-gun captured bighorn sheep then usual basal values in a stress prone swine species (Gronert and Theye, 1976). As seen in Table 1, CK and glucose values were compatible with those previously reported for net-gun captured bighorn sheep (Kock et al., 1987a). Mean rectal temperature was 40.6 C at the time of sampling in base camp; at time of capture, it was 40.8 C.

DISCUSSION

Acid-base reference data on normal unexcited unrestrained bighorn sheep are not available and are unattainable with present methodologies. Data from unexcited domestic Suffolk sheep do not provide a direct comparison (Table 2), but are representative of baseline acid-base values in a variety of species, including racing greyhounds (Ilkiw et al., 1989), mongrel dogs (Michenfelder and Theye, 1968), humans, and the stress-susceptible pig (Gronert and Theye, 1976). Thus the bighorn sheep likely has resting values in this same range. Venous pH and PCo_2 values from captured bighorn sheep reflect a state of metabolic acidosis. A probable major contributing factor to this state is the accumulation of lactic acid in the body resulting from anaerobic glycolysis during pursuit and capture. Harthoorn and Young (1974) have previously commented on acidosis that occurs during wild animal capture. This pattern is expected after a period of intense exercise, and remarkably similar to changes seen in racing greyhounds (Ilkiw et al., 1989).

Reversal of this base deficit would be as follows: body weight, Kg × 0.3 × deficit = needed bicarbonate in mEq. Generally, 50% of this amount is given rapidly, with further treatment depending upon individual responses. Whole body buffering systems are dynamic, and endogenous sta-

TABLE 2. Blood gas, catecholamine values, and body temperature of normal domestic sheep.

	Number	Mean	Standard error	Range
Venous blood gas values				
pH	5	7.43	0.02	7.41–7.47
PCo_2 (k Pa) (mm Hg)	5	5.2 (39)	0	4.5–5.3 (34–40)
PO_2 (k Pa) (mm Hg)	5	5.3 (40)	2	4.3–6.0 (32–45)
Base excess (mEq/l)	5	2	1	−2–5
Catecholamine				
Norepinephrine (ng/ml)	18	0.55	0.12	0.03–1.95
Epinephrine (ng/ml)	18	0.28	0.07	0.05–1.05
Dopamine (pg/ml)	17	67	17	8–260
Body temperature				
Rectal temperature (C)	17	39.6	0.1	39.0–40.8

bilization of pH occurs as stresses are lessened, if perfusion is adequate. Further, exogenous bicarbonate combines with lactate to produce CO_2, and significant additional CO_2 must then be exhaled (Bishop and Weisfeldt, 1976). In primates, physical restraint and needle stick can cause a shift in acid-base balance (Munson et al., 1970). This may have influenced the values recorded from these bighorn sheep.

Bighorn sheep values cannot be directly and statistically contrasted to those in domestic sheep, but a comparison is strengthened by data from other species (Adrogué et al., 1989; Michenfelder and Theye, 1968; Gronert and Theye, 1976). Jugular venous PO_2 values were apparently increased in recently captured bighorn sheep. As compared to values from calm domestic sheep (Table 2), since jugular venous blood reflects metabolic activity of the head, face, and neck, the increase in venous PO_2 suggested an increase in blood flow with stable and unchanged oxygen extraction at time of sampling (Adrogué et al., 1989).

Catecholamine levels (Table 1) do not directly reflect sympathetic nervous activity, but they are an indirect indication of stress responses (Weissman, 1990). Norepinephrine and epinephrine levels were nearly four times higher than those seen in domestic sheep (Table 2). These changes are less than those seen in malignant hyperthermia (MH) in susceptible pigs (Gronert and Theye, 1976). The MH stress syndrome has been reported in man and pigs, and has been compared to capture myopathy (CM) in wild animals (Haigh, 1987).

In conclusion, venous acid-base balance in recently captured bighorn sheep demonstrates a severe metabolic acidosis (base deficit at least 20 mEq/l), due to helicopter-supported capture and manual restraint during blood sampling. Catecholamine levels indicate significant stress, but its degree is not measurable in this study. None of the 47 bighorn sheep captured in this work died of acute capture myopathy, and none showed signs of myopathy upon release. No mortalities were discovered during radio telemetry surveys in the first 2 wk following capture.

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