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PHYSIOLOGIC RESPONSES OF GRIZZLY BEARS TO DIFFERENT METHODS OF CAPTURE

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ABSTRACT: The physiologic effects of two methods of capture, chemical immobilization of free-ranging (FR) bears by remote injection from a helicopter and physical restraint (PR) by leg-hold snare prior to chemical immobilization, were compared in 46 grizzly bears (*Ursus arctos*) handled during 90 captures between 1999 and 2001. Induction dosages and times were greater for FR bears than PR bears, a finding consistent with depletion of, or decreased sensitivity to, catecholamines. Free-ranging bears also had higher rectal temperatures 15 min following immobilization and temperatures throughout handling that correlated positively with induction time. Physically restrained bears had higher white blood cell counts, with more neutrophils and fewer lymphocytes and eosinophils, than did FR bears. This white blood cell profile was consistent with a stress leukogram, possibly affected by elevated levels of serum cortisol. Serum concentrations of alanine aminotransferase, aspartate aminotransferase, and creatine kinase were higher in PR bears that suggested muscle injury. Serum concentrations of sodium and chloride also were higher in PR bears and attributed to reduced body water volume through water deprivation and increased insensible water loss. Overall, different methods of capture resulted in different patterns of physiologic disturbance. Reducing pursuit and drug induction times should help to minimize increase in body temperature and alteration of acid-base balance in bears immobilized by remote injection. Minimizing restraint time and ensuring snare-anchoring cables are short should help to minimize loss of body water and prevent serious muscle injury in bears captured by leg-hold snare.

Key words: Capture, chemical immobilization, grizzly bear, leg-hold snare, physical restraint, physiologic effects, stress.

INTRODUCTION

In many situations, capture and handling wildlife imposes stress, a normal adaptive response in which the target animal uses energy to cope with some threat to its welfare. However, when a threat is extreme or prolonged, the stress response can have a deleterious effect on an animal's health and result in a physiologic state known as "distress" (Moberg, 1999). In distress, energy is used at the expense of other biologic functions including reproduction, tissue growth and maintenance, or immune response and, if unchecked, can result in death. The consequences of capture-related stress have implications for both wildlife health (Kock et al., 1987b; Spraker, 1993; Douglass et al., 2000; Haulton et al., 2001; Jessup, 2001) and interpretation of research results (St. Aubin and Geraci, 1989; Hellgren et al., 1993; Huber et al., 1997). Understanding the physiologic responses to different methods of capture and handling enables appropriate selection of methods to minimize the amount of stress imposed on animals, and to reduce the risk of distress or death at the time of capture and in the days that follow.

Here, data are presented comparing physiologic effects of two methods of capture on wild grizzly bears (*Ursus arctos*). With one method, free-ranging bears were located from a helicopter and immobilized

by remote injection. With the other method, bears were captured and restrained by leg-hold snare for as long as 24 hr prior to chemical immobilization.

MATERIALS AND METHODS

Forty-six free-ranging grizzly bears were handled during 90 captures that occurred in west-central Alberta (Canada; 52°40′–53°60′N, 116°50′–118°00′W) between April 1999 and August 2001 as part of the Foothills Model Forest Grizzly Bear Research Project. For 41 captures, free-ranging (FR) grizzly bears were located by helicopter and immobilized using remote injection (Pneudart® Inc., Williamsport, Pennsylvania, USA and Paxarms® N.Z. Ltd., Timaru, New Zealand) with combinations of zolazepam and tiletamine (ZT; Telazol®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) at 8–10 mg/kg estimated body weight, or xylazine-zolazepam-tiletamine (XZT; Cervizine 300®, Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA) administered as xylazine at 2 mg/kg and Telazol® at 3 mg/kg. For 49 captures, grizzly bears were first captured and physically restrained (PR) by spring-activated leg-hold snare (Margo Supplies Ltd., High River, Alberta) for as long as 24 hr before immobilization with ZT or XZT. The capture and handling protocol was approved through the Animal Care Committee at the University of Saskatchewan (Saskatchewan, Canada; protocol number 19990023).

Pulse and respiratory rates, and rectal temperature (Excel 10® digital thermometer, AMG Medical, Montreal, Quebec, Canada), were recorded for all bears at onset of handling and every 15 min afterwards during the 75 min of handling. Blood was drawn from the medial saphenous vein into sterile tube for biochemical analysis, and into an ethylenediaminetetraacetic acid (EDTA) tube for hematology. Blood samples for serum biochemistry were centrifuged and the serum extracted and stored frozen (−18 C) until laboratory analysis within 1 mo using a biochemistry analyzer (Abbott Spectrum® Series II, Abbott Laboratories Diagnostic Division, Abbott Park, Illinois, USA). Blood samples in EDTA were chilled and analyzed for complete blood cell profiles within 24 hr using a hematology analyzer (Abbott Cell-Dyn® 3200, Abbott Laboratories Diagnostic Division). To determine actual drug dosages, bears were weighed in a sling suspended beneath a load scale (MSI-7200 Dynalink, Precision Giant Systems Inc., Edmonton, Alberta, Canada).

All data were analyzed using SPSS® 10.0 for Windows® (SPSS Inc., Chicago, Illinois, USA). Two-way ANOVA was used to compare induction features, physiologic measures, hematology, and serum biochemistry between methods of capture (FR vs. PR) and between drugs (Zar, 1996). Julian date of capture and age of bear in years were included as covariates for all analyses. Where assumptions of parametric statistics were violated, data were transformed to their natural logarithm and analyzed accordingly. Statistical significance was assigned when the probability (P) of a type I error was ≤ 0.05. All results are reported as the mean ± standard error (SE).

RESULTS

Although induction dosages (mg/kg based on actual body weight) did not differ statistically between free-ranging bears immobilized by remote injection from a helicopter (FR) and bears that were captured and physically restrained by spring-activated leg-hold snare (PR) prior to chemical immobilization, dosages tended to be higher in FR bears (8.70±0.80 mg/kg vs. 7.79±0.69 mg/kg; $F=2.79$, $P=0.10$). Induction times also were greater in FR bears than in PR bears (6.64±0.62 min vs. 5.18±0.47 min; $F=4.23$, $P\leq 0.05$).

Pulse and respiratory rates at 15 min following immobilization were similar between groups (pulse rate: $F=0.14$, $P=0.71$; respiratory rate: $F=1.26$, $P=0.27$), but rectal temperatures were higher in FR bears (38.9±0.22 vs. 38.0±0.15, $F=9.13$, $P\leq 0.01$). Rectal temperatures throughout the handling period also were significantly correlated with induction time in FR bears (post-injection times: 15 min—$r=0.60$, $P\leq 0.01$, $n=28$; 30 min—$r=0.36$, $P\leq 0.05$, $n=38$; 45 min—$r=0.45$, $P\leq 0.05$, $n=39$; and 60 min—$r=0.77$, $P\leq 0.001$, $n=21$).

The proportions of white blood cell subpopulations were affected by method of capture. White blood cell counts (WBC) and neutrophil proportions were higher in PR bears than in FR bears (WBC: $12.2\pm0.74\times10^9$/l vs. $6.9\pm0.46\times10^9$/l, $F=33.0$, $P\leq 0.001$; neutrophils: 90±0.9% vs. 75±2.0%, $F=48.7$, $P\leq 0.001$). Conversely, proportions of lymphocytes and eosinophils were lower in PR bears (lymphocytes: 5±0.6% vs. 16±1.4, $F=46.9$,

$P \leq 0.001$; eosinophils: 0.8±0.14% vs. 3.4±0.50%, $F=19.5$, $P \leq 0.001$).

Numerous serum biochemistry values were affected by method of capture. Serum concentrations of sodium and chloride were higher, and concentrations of potassium and calcium and the anion gap were lower, in PR bears than in FR bears (sodium: 144±0.7 mmol/l vs. 139±0.8 mmol/l, $F=26.6$, $P \leq 0.001$; chloride: 109±1.1 mmol/l vs. 102±1.2 mmol/l, $F=15.9$, $P \leq 0.001$; potassium: 3.9±0.10 mmol/l vs. 4.2±0.10 mmol/l, $F=4.9$, $P \leq 0.05$; calcium: 2.31±0.027 mmol/l vs. 2.40±0.031 mmol/l, $F=4.5$, $P \leq 0.05$; anion gap: 21±0.6 mmol/l vs. 23±0.9 mmol/l, $F=6.6$, $P \leq 0.05$). Serum concentrations of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) were higher, and γ-glutamyltransferase (γ-GT) was lower, in PR bears (ALT: 63±5.3 U/l vs. 41±5.9 U/l, $F=5.3$, $P \leq 0.05$; AST: 264±36.0 U/l vs. 125±23.4 U/l, $F=16.6$, $P \leq 0.001$; CK: 2,202±653 U/l vs. 189±29.5 U/l, $F=5.3$, $P \leq 0.05$; γ-GT: 18±1.6 U/l vs. 29±4.3 U/l, $F=9.3$, $P \leq 0.01$). Further, glucose and total cortisol concentrations were higher, and creatinine concentration was lower, in PR bears (glucose: 8.0±0.35 mmol/l vs. 7.3±0.56 mmol/l, $F=6.8$, $P \leq 0.01$; total cortisol: 222±25.3 nmol/l vs. 147±16.6 nmol/l, $F=4.7$, $P \leq 0.05$; creatinine: 90±4.7 μmol/l vs. 130±8.0 μmol/l, $F=12.8$, $P \leq 0.001$). Among all bears, γ-GT concentration was correlated with rectal temperature at 15 min following immobilization ($r=0.27$, $P \leq 0.05$, $n=76$).

Although induction features, physiologic measures, and blood values also were affected by immobilizing drug, these results are presented elsewhere (Cattet et al., 2003).

DISCUSSION

Chemical immobilization of free-ranging (FR) grizzly bears by remote injection from a helicopter resulted in longer induction times and tended to require higher drug dosages than did chemical immobilization of bears captured and physically-restrained (PR) by leg-hold snare. In general, high circulating levels of catecholamines (epinephrine and norepinephrine) modify the effects of immobilizing drugs and result in delayed induction of immobilization and increased drug requirement or, in some situations, ineffective immobilization (Fowler, 1995; Kreeger, 1996; Nielsen, 1999). In laboratory rats, prolonged physical restraint of 30–240 min results in progressive depletion of catecholamine stores (Dronjak et al., 1999) and decreased sensitivity to catecholamines (Satoh, 1998). Although catecholamine levels were not determined in this study, extending these results to grizzly bears would suggest catecholamine activity was lower in PR bears than in FR bears as a result of the stress associated with a prolonged period of physical restraint prior to chemical immobilization. As a result, PR bears were more sensitive to the effects of immobilizing drugs than were FR bears. In this study, continual effort was made to reduce the duration of physical restraint experienced by bears captured in snares, e.g., deployment of trap transmitters, frequent site visits, etc. Nevertheless, the remote locations of some sites limited the frequency of site visits to once per 24 hr, therefore some bears may have been restrained for as long as 24 hr prior to chemical immobilization.

Rectal temperatures at 15 min following chemical immobilization were higher in FR bears than in PR bears. Further, induction time was positively correlated with rectal temperatures recorded throughout the handling period. This was likely a result of strenuous activity by FR bears while fleeing from the helicopter in the moments prior to chemical immobilization or while progressively succumbing to the effects of the immobilizing drugs. As well, increases in circulating levels of norepinephrine produce vasoconstriction, which decreases heat loss and leads to a rise in body temperature (Ganong, 1995), a factor that may also have contributed to the higher temperatures in FR bears. Observations

of extensive damage to standing vegetation in the immediate vicinity of leg-hold snare sites indicated intense physical exertion was also characteristic of some PR bears. Nevertheless, many of these bears were chemically immobilized hours following their bout of intense activity and it is likely that sufficient time had elapsed for their body temperature to return toward normal. In addition, snare sites were typically constructed in areas that were well shaded and unlikely to contribute to thermal stress.

Bears captured by leg-hold snare had higher concentrations of white blood cells, higher proportions of neutrophils, and lower proportions of lymphocytes and eosinophils than did FR bears. This pattern of white cell proportions is a typical stress leukogram observed in domestic species following adrenal stimulation or glucocorticoid administration (Feldman et al., 2000). This physiologic stress response was likely mediated by elevated levels of cortisol in the blood of PR bears relative to levels in FR bears. Higher serum concentrations of glucose in PR bears may have also occurred as a result of cortisol-mediated reduction in peripheral utilization of glucose and stimulation of gluconeogenesis (Goldstein et al., 1993; Ganong, 1995). Similar results (stress leukogram and high serum cortisol and glucose concentrations) have also been reported for red foxes (*Vulpes vulpes*) caught by padded-jaw foothold traps relative to foxes captured in box traps (White et al., 1991).

Bears captured by leg-hold snare had higher serum concentrations of ALT, AST, and CK than did FR bears. These enzymes are found in large quantity in muscle and high concentrations of all three enzymes in serum at the same time typically signify degenerative or necrotizing muscle injury (Duncan et al., 1994). Muscle injury in PR bears likely occurred in association with tightening of the snare cable around the distal forelimb and excessive strain on muscles and joints of the forelimb proximal to the closed snare. Elevated levels of muscle enzymes in association with physical capture have also been reported for black bears (*U. americanus*), brown bears, and polar bears (*U. maritimus*) captured by leg-hold snare (Lee et al., 1977; Schroeder, 1987; Huber et al., 1997), and for red foxes captured by foothold trap (White et al., 1991). Although muscle injury may be common when using leg-hold snares, chemical immobilization by remote injection from helicopter may also result in significant muscle injury as indicated by the high levels of muscle enzymes that occurred in some of the FR bears.

Elevated levels of muscle enzymes, potassium, and creatinine in some FR bears may have also occurred as a result of muscle activity during capture. Intense muscular activity can result in release of intracellular potassium, creatinine, and enzymes from muscle cells without any pathologic consequences. In exercising humans, potassium efflux from muscle cells is mediated by catecholamines and results in elevated serum levels for a short time before returning to normal (Williams et al., 1985). Similarly, circulating creatinine can be elevated temporarily by exercise (Refsum and Stromme, 1974). It is unlikely that the higher concentrations of creatinine in FR bears reflected renal dysfunction since urea, which is also cleared by the kidneys, was similar between FR and PR bears. Elevations in serum potassium, creatinine, and muscle enzymes have also been documented in beluga whales (*Delphinapterus leucas*) during capture from the wild (St. Aubin and Geraci, 1989), and in bighorn sheep (*Ovis canadensis*) captured by drop-net or chemical immobilization by remote injection from a helicopter (Kock et al., 1987a).

Serum concentrations of γ-GT were higher in FR bears than in PR bears. Increased levels of γ-GT are typically associated with liver disease, specifically biliary stasis (Meyer, 1983; Duncan et al., 1994). In normal health, however, γ-GT can leak into plasma from other tissues because it occurs as a membrane bound enzyme in a

variety of tissues that include liver, heart, kidney, skeletal muscles, and tissues of the reproductive organs (Viña et al., 1989; Hanigan and Frierson, 1996; Leeuwenburgh et al., 1997). Although the tissue source of γ-GT was not determined for grizzly bears, the higher concentration in FR bears was speculated to occur as a result of increased metabolic activity during pursuit and capture based on the observation that γ-GT concentration was directly correlated with rectal temperature. Elevated levels of γ-GT have also been reported in beluga whales following capture, but in this case were suggested to result from inadequate perfusion and anoxia of liver tissue caused by stress-induced circulatory insufficiency (St. Aubin and Geraci, 1989).

Intense muscle activity immediately preceding chemical immobilization also explained the observation that anion gap and serum calcium concentration were higher in FR bears than in PR bears. The release of energy through anaerobic glycolysis would be expected to result in increased levels of lactic acid, or more generally unmeasured anions, in the circulation. As the anion gap increased, the release of calcium into circulation increased as a physiologic response to offset any acid-base disturbance caused by the accumulation of lactic acid.

Reduced body water volume was the most probable explanation for the higher concentrations of sodium and chloride in PR bears. Bears captured by leg-hold snare were deprived of water for prolonged periods (2–23 hr), and likely had increased insensible water loss associated with the struggle to escape. Although dehydration was mild in most cases, it was significant enough in a few bears to result in moderate elevations (≥mean+2SD) in sodium (≥152 mmol/l), chloride (≥121 mmol/l), urea (≥19.8 mmol/l), and total protein (≥81 g/l). Without the insight provided by serum biochemistry, this level of dehydration (i.e., ≤5% of body weight in fluid loss) would likely go undetected in most captured bears. Nevertheless, intravenous fluid therapy (e.g., lactated Ringer's solution) could be of benefit to some PR bears.

In conclusion, immobilization by remote drug injection from a helicopter and capture and physical restraint by leg-hold snare caused different patterns of physiologic disturbance in grizzly bears. Disturbances observed in bears immobilized by remote injection from a helicopter were increased body temperature and slight alteration of acid-base balance. Reducing pursuit and drug induction times should help to minimize occurrence of these types of disturbances. The main physiologic disturbances in bears captured by leg-hold snare were muscle injury and dehydration. In addition, many bears developed a stress leukogram, likely as a consequence of their longer duration of stress relative to that experienced by free-ranging bears immobilized from a helicopter, i.e., subacute vs. acute stress. Minimizing the time bears are restrained in snares prior to chemical immobilization and ensuring that snare-anchoring cables are kept short should help to minimize loss of body water and prevent serious muscle injury.

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