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Hormonal and Hemodynamic Responses to 15 ml/kg Hemorrhage in Conscious Dogs: Responses Correlate to Body Temperature (41117)

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Abstract. Hemorrhage stimulates the secretion of ACTH, corticosteroids, vasopressin, and renin. Elevated levels of these hormones may be required for the restoration of normal blood volume and pressure. These experiments were designed to quantitate the simultaneous responses of these hormones after 15 ml/kg hemorrhage in conscious dogs. Nine conscious dogs were bled 15 ml/kg from chronically maintained femoral arterial catheters. In 20 experiments, the hormonal and hemodynamic responses to the hemorrhage were not reproducible from dog to dog or from experiment to experiment in dogs studied more than once. The dogs' rectal temperatures ranged from 38.2 to 41°, although nearly all appeared normal and healthy at the time of study. Most of the variability in the data could be explained on the basis of these differences in rectal temperatures. The dogs with elevated rectal temperatures (39–41°) had higher resting heart rates, ACTH, corticosteroids, vasopressin, and plasma renin activity, and greater blood pressure, ACTH, corticosteroid, and vasopressin responses than normothermic dogs (rectal temperatures 38–39°). We conclude that (i) not all dogs that appear to be healthy respond to hemorrhage equally; (ii) the variability of hormonal and hemodynamic responses to hemorrhage in a random sample of conscious dogs can be reduced by preselection of animals on the basis of rectal temperature.

Hemorrhage initiates a series of homeostatic endocrine responses including increased circulating levels of ACTH (1), adrenocortical steroids (2, 3), vasopressin (4), and plasma renin activity (5). Using pentobarbital-anesthetized dogs, Gann (2) has shown that the magnitude of the adrenal venous corticosteroid response to hemorrhage is proportional to the logarithm of the volume of blood removed (threshhold dose 2–3 ml/kg), and is suppressed by prior administration of dexamethasone (when the hemorrhage volume is less than 20 ml/kg). Based on the results of these studies, we chose to apply a 15 ml/kg hemorrhage in conscious dogs to study cortisol feedback on stimulus-induced ACTH secretion.

However, we found that 15 ml/kg hemorrhage induced highly variable cardiovascular and hormonal responses whose magnitudes were unrelated to the time or dose of cortisol infusion. We therefore changed our objectives and studied the variability in the magnitudes of the cardiovascular and hormonal responses to hemorrhage, and defined rectal temperature as a measured covariate of response magnitude. The results of this study demonstrate the importance of using rectal temperature as an objective criterion for assessing animals' suitability for use in studies of hormonal responses to hemodynamic stimuli.

Some of these data have been reported in abstract form (6).

Materials and Methods. We studied nine mongrel dogs of either sex weighing between 15 and 30 kg. They were housed in individual cages and given free access to food and water. Before surgery, the dogs were accustomed to standing in a loose cloth sling (Alice King Chatham Medical Arts, Inc.).

Surgery. Dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv), and under sterile conditions a catheter was inserted into a femoral artery, and the tip was advanced to the common iliac artery. Catheter material was either Medical Grade Silastic tubing (Dow Corning, Inc., 0.078-in. i.d., 0.125-in. OD) or Tygon R-3603 polyvinylchloride tubing (Norton Plastics, Inc., 0.094-in. i.d., 0.156-in. OD). The cannula was routed subcutaneously from a femoral triangle to the back where

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the free end emerged through the skin. The catheter was filled with sodium heparin (1000 units/ml) and the end was wrapped in a clean gauze packet which was held in place by a zippered jacket (Alice King Chatham Medical Arts, Inc.). At least 2 days elapsed between surgery and experimentation.

Experiments. All experiments were started between 0800 and 1000 hr to minimize between-dog variation due to possible circadian variations in resting hormone levels and hormone response to hemorrhage. On the morning of an experiment, a dog was brought from the animal quarters to the laboratory, placed in the sling, and its rectal temperature was measured using a clinical mercury rectal themometer, inserted to a depth of 6 cm and held in place for at least 2 min. The thermometer was read to the nearest 0.1°. The ambient of temperature in the laboratory is regulated between 19 and 22°. Next, a superficial leg vein was catheterized (Angiocath, 18 g, Deseret Pharmaceutical Co.).

Infusion. In five experiments on five dogs, cortisol (17 μg/min) was infused from 120 to 60 min before the start of hemorrhage. The rectal temperatures of these dogs ranged from 38.8 to 40.9°. In four experiments, a saline infusion (from 120 to 60 min before hemorrhage) was administered. All saline used in these experiments was sterile, pyrogen-free normal saline (Travenol Laboratories, Inc.). In 11 experiments, dogs were not infused and received venipuncture at least 40 min before hemorrhage.

Hemorrhage and sampling. Twenty hemorrhage experiments were performed on the nine dogs; three were studied once, three twice, one three times and two dogs were studied on four occasions. At least 2 days were allowed between experiments on each dog studied more than once. Blood was pumped from the femoral arterial catheter using a roller pump at a rate sufficient to achieve a 15 ml/kg hemorrhage within 3 min. The hemorrhage volume was pumped into a sterile Donor-Pack (Fenwal Co.) which contained 1000 units of sodium heparin. Thirty minutes after the onset of hemorrhage, the shed blood was returned to the dog over a period of 5 min. Twelve femoral arterial blood samples (8–12 ml) were collected for hormone analysis. Heart rate and femoral arterial blood pressure were recorded using a Statham pressure tranducer and a Grass polygraph. At the end of all experiments, the red blood cells from blood samples were resuspended in sterile, pyrogen-free normal saline and returned to the dog.

Hormone assay. Blood samples were placed on ice, centrifuged at 4°, and plasma was assayed for ACTH, corticosteroids, vasopressin, and renin activity. Plasma vasopressin was measured by radioimmunoassay (7), as was ACTH (8–10). All ACTH samples from a single experiment on one animal were run in a single extraction and incubation. We tested the reproducibility of the assay in the range of ACTH values of interest by running in one assay multiplicate samples of a plasma pool using both 1.0-ml aliquots and 0.5-ml aliquots diluted to 1.0 ml in ACTH-free plasma. Mean values for five samples of 1.0 ml were 65 ± 4.9 (SD), and for four samples of 0.5 ml were 31.0 ± 2.9 (SD) pg ACTH/sample. Thus, at these levels, the intraassay coefficient of variation of the assay is 8–9%. Over a period of 3 months, the interassay coefficient of variation was 19% for a plasma pool that contained an average of 78 pg ACTH/ml (N = 46 extractions and assays).

Plasma renin activity was measured using a radioimmunoassay for angiotensin I generated during a 3-hr incubation *in vitro* (11, 12). Plasma corticosteroids were measured using a competitive protein binding assay (13), employing human transcortin as the binding protein.

Plasma sodium concentration was measured using an Instrument Laboratories flame photometer. Plasma osmolality was measured using an Advanced osmometer.

Statistical analyses. Data were analyzed using paired t test, linear regression analysis, and one-way analysis of variance for repeated measures.

Results. In 20 experiments on nine conscious dogs, rapid 15 ml/kg hemorrhage

(achieved within 3 min) and 30 min of the resultant hypovolemia decreased blood pressure, increased heart rate, and increased levels of plasma ACTH, corticosteroids, vasopressin, and renin activity (Table I; all $P < 0.001$ by one-way analysis of variance for repeated measures). However, the hormonal and hemodynamic responses to the hemorrhage were quite variable from dog to dog, and from experiment to experiment in each dog that was studied more than once.

The dogs' rectal temperatures at the time of study ranged from 38.2 to 41°, although nearly *all* dogs appeared to be normal and healthy (bright eyes, energetic, wagging tail, etc.). One of the nine dogs (in 2 of 20 experiments) appeared depressed and was found to have rectal temperatures of 40.9 and 41°. The range of normal body temperature in dogs is 38–39° (14). Dogs with elevated rectal temperatures had higher resting heart rates and higher prehemorrhage plasma ACTH, corticosteroids, and vasopressin than normothermic dogs (Table II). The dogs that were infused with cortisol (indicated on the figure by enclosed symbols) were fairly evenly distributed across the 38.2–41.0° rectal temperature range. The number of dogs in this group was small; however, the results suggest that the prior infusion may have affected the magnitudes of the ACTH, 11OHCS and vasopressin responses.

Plasma Na^+ concentrations were measured in five dogs whose rectal temperatures increased from 38.9 ± 0.2 to 40.7 ± 0.2° between the first and second experiment. Prehemorrhage sodium concentration fell from 143 ± 0.2 to 137 ± 0.6 mEq/liter ($P < 0.001$ by paired t test). There was no significant coincident change in the dogs' body weights (change in weight = −0.3 ± 0.4 kg; P = NS by paired t test). Plasma osmolality was measured in three of these five dogs and decreased from 292 ± 1 to 279 ± 2 mosm/liter as the dogs became febrile.

Discussion. The results of this study demonstrate that there is considerable variability in the magnitudes of hormonal and hemodynamic responses to hemorrhage in conscious dogs when the dogs are randomly selected for study, and that much of this variability can be correlated to rectal temperature at the time of the experiment. We do not conclude from these data that body temperature per se controls or directly influences the hormone response to 15 ml/kg hemorrhage in conscious dogs. However, dogs with fevers (probably of differing etiologies) respond to hemorrhage predictably: the magnitudes of the ACTH, corticosteroid, and vasopressin responses to

TABLE I. HEMODYNAMIC AND ENDOCRINE RESPONSES TO 15 ml/kg HEMORRHAGE IN CONSCIOUS DOGS

Time[a]	$\bar{P}$[b] (Torr)	hr[b] (min^{-1})	ACTH[b] (pg/ml)	11OHCS[b] (μg/dl)	PRA[b] (ng Al/ml/3hr)	ADH[b] (pg/ml)
−10	97 ± 3	87 ± 9	37 ± 5	2.2 ± 0.3	4.2 ± 0.4	6 ± 1
− 2	99 ± 3	92 ± 8	38 ± 6	2.6 ± 0.4		
0	94 ± 3	92 ± 9	41 ± 7	2.5 ± 0.4	4.4 ± 0.5	6 ± 1
2	61 ± 10	115 ± 14				
4	84 ± 6	99 ± 7	119 ± 29	3.2 ± 0.8		
6	81 ± 4	109 ± 7	130 ± 31	4.5 ± 0.9	8.1 ± 1.0	166 ± 48
8	78 ± 4	111 ± 8	130 ± 29	4.3 ± 0.8		
10	75 ± 4	112 ± 9	142 ± 31	5.8 ± 0.9	8.0 ± 1.1	127 ± 35
15	78 ± 5	113 ± 6	161 ± 36	6.2 ± 0.9	7.2 ± 1.1	164 ± 48
20	75 ± 4	117 ± 7	159 ± 32	7.7 ± 1.1	7.2 ± 0.8	169 ± 57
25	74 ± 4	123 ± 9	181 ± 37	7.9 ± 1.1	7.8 ± 1.0	150 ± 59
30	73 ± 3	127 ± 9	187 ± 38	7.6 ± 1.0	8.4 ± 1.0	109 ± 35
60	100 ± 4	88 ± 8	67 ± 9	4.2 ± 0.6	5.0 ± 0.5	20 ± 4

Note. Rectal temperatures ranged from 38.2 to 41°.

[a] Dogs were bled 15 ml/kg between 0 and 3 min. Blood was returned at 30 min.

[b] N = 20 experiments in nine dogs.

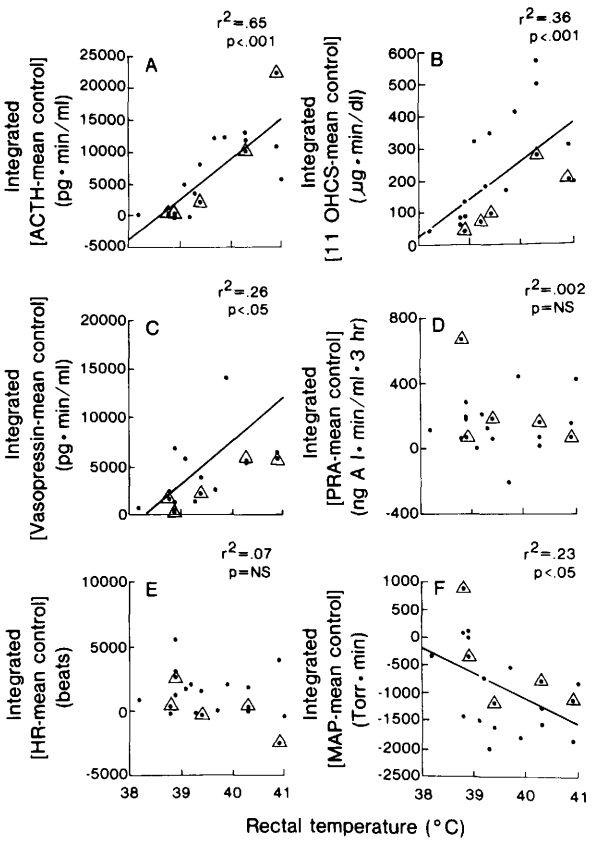

FIG. 1. Correlation of integrated hormonal and cardiovascular responses to hemorrhage with rectal temperature at the time of the experiment. The integrated response (ACTH (A), corticosteroids (B), vasopressin (C), plasma renin activity (D), heart rate (E), and mean femoral arterial blood pressure (F)) represents the area under the entire curve obtained over 70 min minus the area over 70 min of the mean value for prehemorrhage samples. There were significant linear relationships between rectal temperature and integrated ACTH, 110HCS, vasopressin, and mean arterial pressure responses to hemorrhage. Results from experiments on dogs that were infused with cortisol (17 μg/min from −120 to −60 min) are enclosed in triangles.

hemorrhage are linearly related to the dog's rectal temperature at the time. These data demonstrate the usefulness and importance of using rectal temperature as an objective criterion in assessing an experimental animal's health and suitability for study. We have found no other published studies of the hormonal responses to hemorrhage in either conscious or anesthetized dogs in which the animals' rectal temperatures were reported.

Most dogs in this study, regardless of rectal temperature, appeared normal and displayed *no* outward signs of sickness at the time that they were studied. Since we have begun routinely measuring rectal temperatures of dogs received at our animal-care facility from pounds elsewhere, we find that at least 30% of our animals arrive at the facility with rectal temperatures above 39°. Other animals develop respiratory infections and fever after they have been at the facility for 1 to 2 weeks. Some dogs which were not febrile before surgery developed fevers after implantation of the arterial catheter. We believe that some of the dogs expressed latent viral infections after surgery. Our subsequent experience has been that if we keep each dog in the animal care facility for at least 4 weeks and "con-

dition" it by treating illnesses that develop during this period, it does not develop an elevated rectal temperature after surgery.

Increased resting- and stimulated-hormone levels in febrile dogs might be explained by decreased blood volume. ACTH, corticosteroids, renin, and vasopressin act directly or indirectly to increase blood volume and therefore mean arterial pressure. If blood volume decreases with the development of fever in our dogs, levels of these hormones might be expected to correlate positively with rectal temperature, and the nominal stimulus of 15 ml/kg hemorrhage would be effectively greater at elevated rectal temperature. Thus, dogs with elevated rectal temperatures have higher resting levels of ACTH, corticosteroids, vasopressin, and renin than normothermic dogs (Table II), and they respond to the hemorrhage with greater decreases in blood pressure and greater increases in ACTH, corticosteroids, and vasopressin than normothermic dogs (Fig. 1). The elevated resting vasopressin concentration might account for the hypoosmolality and hyponatremia observed in the dogs with elevated rectal temperatures. Interestingly, the renin response to the hemorrhage is not greater in the dogs with elevated rectal temperatures than in the normothermic dogs (Fig. 1). It is possible that in the dogs with elevated rectal temperatures, the large increases in vasopressin during hypovolemia might be inhibiting the renin response. Vasopressin has been shown to inhibit renin secretion in sodium-depleted (15) and sodium-replete (16) dogs.

In summary, we cannot conclude that elevated body temperature is itself changing the hormonal responses to hemorrhage; however, it seems likely that another factor coupled to or associated with body temperature (blood volume?) is responsible for altering the endocrine and hemodynamic responses to hypovolemia. However, because febrile dogs often appear quite normal and healthy, investigators should use an objective criterion, such as body temperature, when they choose animals for use in studies of hormonal responses to hemodynamic stimuli.

TABLE II. CORRELATIONS OF BASELINE VALUES WITH RECTAL TEMPERATURE

Correlated variable	*r*	*P*	*N*
ACTH	+0.54	<0.005	20
11OHCS	+0.70	<0.001	20
Vasopressin	+0.47	<0.05	20
PRA	+0.41	<0.05	20
MAP	−0.26	NS	20
HR	+0.44	<0.05	20

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