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CHARACTERIZATION OF BLOOD DRAWN RAPIDLY FOR USE IN BLOOD VOLUME EXPANSION STUDIES: AN ANIMAL MODEL FOR SIMULATED WEIGHTLESSNESS

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This study demonstrates that up to 8ml of blood can be drawn from donor rats without significantly increasing volume and stress sensitive hormones and thus, can be used for volume expansion studies. Infusion of whole blood allows more physiological changes than can be seen with volume expansion by saline or other ionic solutions. Furthermore, the infusion of whole blood to induce hypervolemia may provide an improved model to study the fluid balance and control mechanisms operative in weightlessness. In this study, blood samples were drawn as rapidly as possible from femoral artery catheters (microrenathane) chronically implanted in Sprague Dawley rats and analyzed for hematocrit, plasma sodium, potassium, osmolality, corticosterone, epinepherine, norepinephrine and vasopressin. The levels were found to be comparable to those of normal rats. Future studies of simulated weightlessness utilizing compatible and physiologically comparable whole blood will provide data that can be used to develop protective measures for the debilitating effects of space flight.

INTRODUCTION

Blood volume expansion studies have been conducted to examine fluid adaptation and cardiovascular control mechanisms using numerous fluids including hypertonic, hypotonic and isotonic saline, water, dextran solution, albumin and polyethylene glycol (references 1,2). Less frequently, investigators have used whole blood, plasma or packed red blood cells from compatible donors (references 3,4). Infusion of whole blood allows the composition of the recipient's blood to remain relatively unaltered. Infusion of saline or other solutions may cause intracompartmental fluid shifts that modify the normal physiological adaptive processes. To prevent the introduction of aberrant hormones and ions that may alter or skew the adaptation processes, the blood infused must be as close as possible in composition to that of the recipient. It is well known that surgery, handling, restraint, injections and commonly used blood sampling techniques can cause fluctuations and changes in the levels of plasma catecholamines and other stress and volume dependent hormones, such as ACTH, renin, corticosterone and prolactin (references 5-8). However, little work has been done to determine at what point in time and/or at what quantity of blood drawn, the blood levels of the volume and stress sensitive hormones change significantly.

In these studies, blood samples were drawn as rapidly as possible from femoral artery cannulated unanesthetized rats. The blood samples were analyzed for hematocrit, plasma sodium, potassium, osmolality, corticosterone, epinephrine, norepinephrine and vasopressin. The data were analyzed in regard to the volume drawn to examine the effects of blood sampling on the levels of hormones and electrolytes listed above.

METHOD

Male Sprague Dawley rats weighing between 350-400 grams were obtained from Charles River Laboratories (Raleigh, NC). All the rats were housed in single cages and maintained on a 12:12 hour light:dark cycle, with the lighted phase beginning at 6 a.m. They were used in the studies when they achieved a minimum body weight of 515 grams, previously determined to be an optimum size for cannulation. Standard rat chow and water were available ad libitum throughout the experiment. Room temperature and humidity were controlled as specified by the National Institutes of Health Animal Care Guide (reference 9).

The rats were anesthetized using a 1:1 mixture of KETASET (ketamine hydrochloride- Aveco Co., Inc.) and GEMINI (xylazine- The Butler Co.) for the cannulation procedure. The cocktail was injected (0.1ml/100gr) intramuscularly (references 10,11). Microrenathane (i.d. 0.025" o.d. 0.040"- Braintree Scientific, Inc.) catheters were inserted into the femoral artery in the right leg of 17 rats and from there led subcutaneously under the skin to emerge from the back of the neck. Microrenathane has been reported to reduce the probability of intravascular thrombosis (reference 12). The catheters were filled with heparin to maintain patency, heat sealed and coiled in a protective stainless steel button on the animal's back. Immediately after surgery, the animals received 0.1ml/100gr injection of DI-TRIM (trimethoprim sulfadiazim, Syntex Animal Health) subcutaneously, as a prophylactic agent against infection (reference 13). The rats were allowed to recover from the surgery for one week. During

this week, they were conditioned to the manipulation of their catheters by daily handling, in the morning. This has been reported to prevent the stress-induced release of corticosterone (reference 14). Non-specific stressful stimuli (i.e., sudden or loud noises, excessive movement, unnecessary handling) were kept to a minimum during the experiment.

At the end of the recovery period, blood samples (3ml samples, n=4; 6ml samples, n=7 or 8ml samples, n=6) were drawn as quickly as possible from conscious animals. The blood samples were obtained between 9 a.m. and 2 p.m. to avoid the effects of circadian variations (reference 15). Prior to obtaining the sample for the assays, the heparin solution filling the dead space of the cannula (about 0.25ml) was removed. Approximately one-third of each of the blood samples was mixed with the anticoagulant EGTA (Ethylene glycol-bis tetraacetic acid) for the catecholamine assay specimen. Approximately 150ul was used to fill three heparinized microhematocrit tubes and the remaining portion of whole blood was mixed with lithium heparin for the other assays. The blood was centrifuged immediately (1500 rpm) at 4°C. The EGTA and heparinized plasma specimens were removed from the red blood cells and frozen in separate microcentrifuge tubes at -20°C until assayed.

The hematocrit was assayed using a previously described microhematocrit procedure (reference 15). The plasma osmolality was determined using vapor pressure osmometry (reference 16). The plasma electrolytes (sodium and potassium) were assayed by flame photometry (reference 17). The catecholamines (epinephrine and norepinephrine were analyzed using a radioenzymatic assay method (reference 18). The other hormones, corticosterone and arginine vasopressin, were determined using a radioimmunoassay method (references 19,20).

Statistics

The data was analyzed in two different ways. Analysis of the statistical difference between the 3ml, 6ml and 8ml samples was performed by one-way analysis of variance. The Mann Whitney Confidence Interval and Test was used to compare each of the blood parameters and the blood sampling time for the 3ml blood sample to the 6ml and the 8ml blood samples individually, since the 3ml sample was the best control for these experiments. Differences were considered significant when $p < 0.05$ (reference 21).

RESULTS

One way analysis of variance was used to determine the statistically significant difference between each of the blood parameters for the volumes of blood obtained. The F test was calculated at a 95% confidence interval for the mean differences at each volume collected (3ml, 6ml 8ml) for each of the blood assays (hematocrit, plasma sodium, potassium, epinephrine, norepinephrine, osmolality, corticosterone and vasopressin) and the time required to obtain the volume. The observed F for each of the blood parameters was less than the critical F (19.4) determined from the F table [hematocrit: $F(2,16) = 0.13$, osmolality: $F(2,16) = 0.29$; sodium: $F(2,15) = 5.26$; potassium: $F(2,15) = 12.51$; corticosterone: $F(2,16) = 0.68$; epinephrine: $F(2,16) = 1.37$; norepinephrine: $F(2,16) = 1.54$; vasopressin: $F(2,16) = 0.07$] including time: $F(2,16) = 2.89$]. Therefore, there is no significant difference between the groups (i.e., blood volumes drawn).

The Mann Whitney Confidence Interval and Test was also computed for the mean differences in the blood parameters listed above. In this analysis, the 3ml blood samples were compared versus first the 6ml blood samples and then versus the 8ml blood samples. The time required to draw the volumes ranged from 17.2 (3ml sample) to 106 seconds (8ml sample). The time required to draw sufficient blood from the donor animals does increase as a function of time, as anticipated. However, there was only a significant difference in the time required to draw the 8ml sample as compared to the 3ml sample, not the 6ml sample. Interestingly, there was a significant difference in the sodium and potassium levels for the 3ml blood samples versus both the 6ml and the 8ml samples (Table 1).

TABLE 1. Blood Volumes Drawn in Respect to Time, Plasma Electrolytes (Sodium & Potassium), Osmolality and Hematocrit Levels.

Volume (ml)	3 (n=4)	6 (n=7)	8 (n=6)	Reference Values
Time (sec.)	28.8 ± 7.26	49.1 ± 7.54	61.3 ± 10.2*	----------
Sodium (mEq/L)	136.5 ± 1.00+	141.9 ± 1.33*	142.7 ± 0.9*	138-148 ($\bar{x}$ =143)
Potassium (mEq/L)	3.9 ± 0.29+	5.01 ± 0.165*	4.39 ± 0.11*	4.0-9.2 ($\bar{x}$ = 6.6)
Osmolality (mosm/kg)	281.9 ± 7.5	277.4 ± 3.37	278.2 ± 2.19	294 ± 1.4
Hematocrit (%)	41.9 ± 3.83	42.57 ± 3.17	41.8 ± 0.93	49 ± 4

Values are ± S.E.M.
Statistical analysis performed using the Mann Whitney U Test.
+ n= 3
* Significant difference compared versus 3ml blood sample, $p < 0.05$

The values were comparable with the range of the reference values reported for normal rats (reference 22). The values for hematocrit, plasma osmolality, epinephrine, norepinephrine, corticosterone and vasopressin measured in the 3ml samples were not significantly different from the 6ml or 8ml samples (see Figures 1,2 and 3).

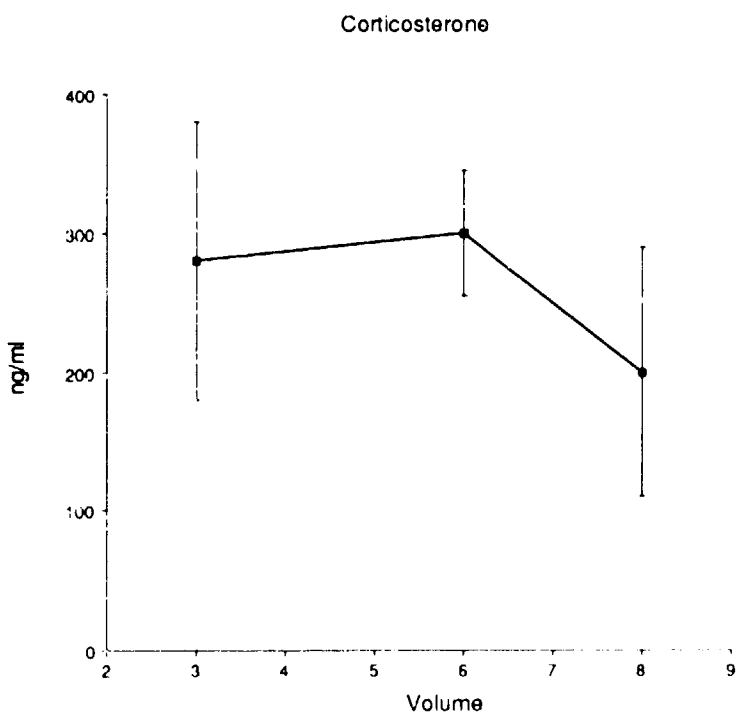

FIGURE 1. Plasma levels of corticosterone in 3ml, 6ml, and 8ml sample volumes. Samples were removed from cannulated animals as quickly as possible (< 2 minutes) and analyzed for corticosterone using a radioimmunoassay. There was no significant difference between the three groups.

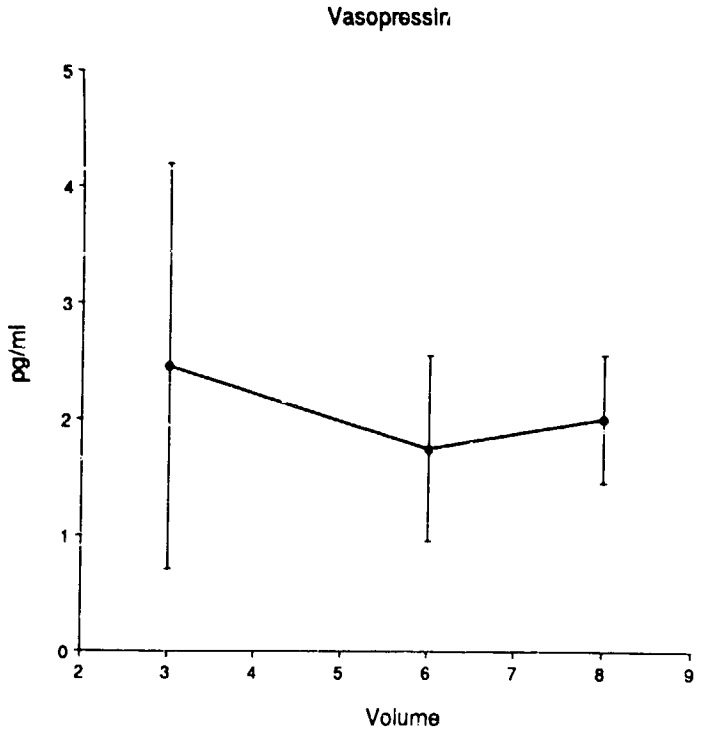

FIGURE 2. Plasma levels of vasopressin in 3ml, 6ml, and 8ml sample volumes. Samples were removed from cannulated animals as quickly as possible (< 2 minutes) and analyzed for vasopressin using a radioimmunoassay. There was no significant difference between the three groups.

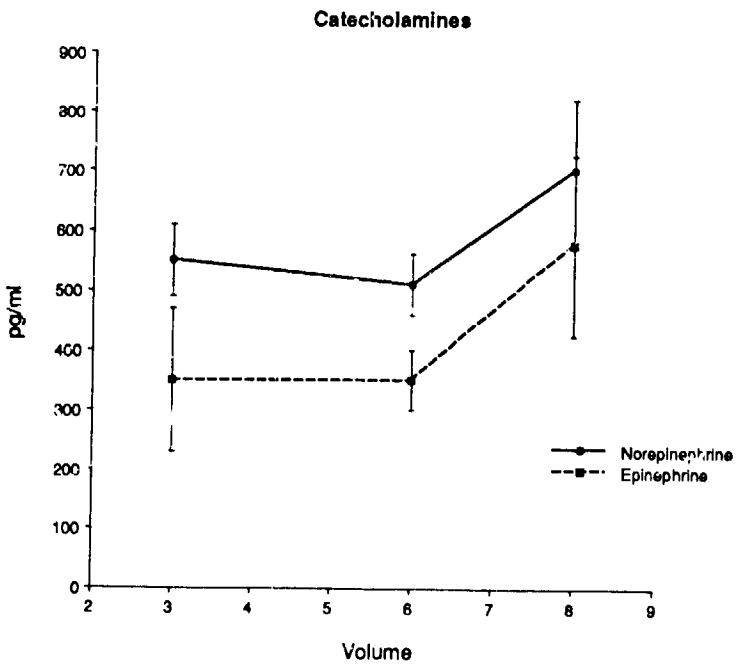

FIGURE 3. Plasma levels of catecholamimes (epinephrine & norepinephrine) in 3ml, 6ml, and 8ml samples volumes. Samples were removed from cannulated animals as quickly as possible and analyzed for the catecholamines using a radioenzymatic assay. There was no significant difference between the three groups.

Furthermore, the values for corticosterone, epinephrine and norepinephrine appear to be within the limits given in other investigations (references 7,8).

DISCUSSION

As expected, the time required to draw blood from donor animals did increase as a function of volume. Surprisingly, there was a significant difference ($p < 0.05$) in the sodium and potassium levels for the 3ml blood samples versus the 6ml and 8ml samples. However, these values were comparable to the reference values observed in blood samples obtained from control animals in other studies (reference 22). The mean hematocrit, osmolality, corticosterone, epinephrine, norepinephrine and vasopressin levels for the 3ml, 6ml and 8ml samples were not significantly different from each other. Moreover, the mean values for the volume and stress sensitive hormones appear to be comparable to the reference levels reported in other studies (references 7,8).

Investigators have studied the numerous and complex adrenocortical and adrenomedullary responses to hemorrhage and transfusion, but, little has been done regarding the plasma electrolyte responses (reference 23). It has been postulated that the electrolyte responses during weightlessness are the result of endocrine changes acting on the kidney to restore "normal" cardiopulmonary volume. The electrolyte response and the endocrine interrelationships need to be clarified.

Plasma catecholamines are a sensitive indicator of sympatho-adrenal medullary activity. The source of the epinephrine has been demonstrated to be predominately from the adrenal medulla (reference 8). The source of the norepinephrine has been demonstrated to be primarily from the sympathetic nerves (reference 8). A recognized component of the immediate physiological response to stress is the release of catecholamines into the blood. Conventional animal manipulations are well known stressors that elicit changes which release catecholamines. The opening of the cage, transfer of animals into another room and short term handling has been reported (reference 24) to cause epinephrine and norepinephrine levels to increase as much as eight fold. In the current studies, withdrawing blood from the femoral artery catheter elicited an increase in the catecholamine levels that correspond with the animal manipulations reported in other studies. Although the values tend to increase concomitant with the volume of blood drawn from the animal, the values for the 3ml samples are not significantly different from the values for the 6ml or 8ml samples.

Corticosterone is secreted by the adrenal cortices of rats and controlled by a complex negative feedback system involving the central nervous system, hypothalamus, pituitary and adrenals (reference 23). Increased plasma corticosterone levels can be induced by the same stress inducing manipulations that increase plasma catecholamine levels (references 7,8). Corticosterone is also a volume sensitive hormone. Studies by Gann in dogs (ref. 23) have demonstrated that plasma cortisol levels are the function of a semi-logarithmic relationship with ACTH and an exponential relationship with the magnitude and rate of the hemorrhage. Other studies have shown that up to 10 minutes is required to allow stimulation of the aforementioned feedback mechanisms to significantly alter the blood levels of corticosterone (reference 25). In the current study, the values for corticosterone in the 3ml samples was not significantly different from the levels in the 6ml or 8ml samples. These samples were drawn in less than 2 minutes, which was not adequate time to stimulate the mechanisms that would significantly influence the corticosterone blood levels.

Arginine vasopressin (AVP) is a hypothalamic hormone, synthesized primarily by hypothalmic neurosecretory neurons whose axons terminate in the pars nervosa of the posterior lobe of the pituitary gland. Vasopressin secretion is controlled by osmotic and nonosmotic factors (reference 26). Vasopressin release is stimulated by a fall in blood pressure, an increase in plasma osmolality or a decrease in plasma volume (reference 27). There is an exponential nature to AVP release in response to hypovolemia, much like the corticosterone response (reference 28). The threshold for stimulation of vasopressin release in hypovolemia is generally to be between 10 and 20% of the blood volume (reference 27). Ginsberg and Brown (ref. 29) studied changes in vasopressin activity after slow and rapid hemorrhages in anesthesized animals and found greater increases in the antidiuretic activity in blood samples drawn more slowly. In the current study, there were no significant differences in the vasopressin levels of the 3ml, 6ml and 8ml samples, which represents hemorrhages of 7.9%, 15.8% and 21% respectively. It can be speculated that in the current study, the degree of hemorrhage or the time for collection was not sufficient to elicit an increase in plasma vasopressin.

This study and the values reported in other studies seem to indicate that "donor" rats' blood values reflect levels that are seen under standard experimental conditions. Therefore, a maximum of 8ml of blood from donor rats can be used for infusion into recipient rats to study the physiological adaptation mechanisms of volume expansion with whole blood.

In space flights, a redistribution of body fluid toward the head and chest as a result of a decrease in hydrostatic pressure in the vasculature of the lower limbs (reference 30) has been observed. As blood from the lower

extremities shifts to the cardiopulmonary space due to loss of gravity, there is an engorgement of the central circulation where the mechanoreceptors involved in plasma volume regulation are located (reference 30). In essence, the body perceives a volume expansion. The short term cardiovascular response mechanisms include an increased venous return, cardiac output, arterial pressure and individual organ blood flow (reference 31,32). The resultant overperfusion of individual organs may bring about long-term autoregulatory changes in arteriolar and venular diameter, number and length (reference 33). The long-term regulatory mechanisms also involve the volume regulating hormones (renin, vasopressin, aldosterone and atrial natriuretic factor). The short-term effects involve the autonomic nervous system and the plasma electrolytes, such as, sodium, potassium and chloride. Alterations are also seen in urine and/or blood levels of kinins, corticosterone and catecholamines (reference 34).

Numerous studies of the effects of microgravity or the weightlessness of space flight have demonstrated that the body's homeostatic mechanisms adapt reasonably soon (days) after exposure to it. Although readaptation also occurs upon return to normal gravity, the process is slow (weeks) and cardiovascular deconditioning (i.e. tachycardia, impaired locomotion, reduced exercise tolerance and orthostatic intolerance) has caused problems for astronauts returning from space (reference 32).

These same changes have been seen in simulations of weightlessness (i.e., bedrest, head-down tilt or immersion) (reference 35). In human and animal studies alike, consistent simulation conditions are difficult to maintain for long periods. Thus, the current data is incomplete, inconsistent and conflicting. Furthermore, the findings during space flight are often difficult to interpret because of the lack of data from the start of launch, reduced possibility to obtain blood samples every day and the lack of simultaneous collection of hemodynamic, hormonal and electrolyte data. It is also difficult to compare the fluid shift data between missions because of the difference between the duration of the flights, nature of the missions and the exercise regime, eating habits and genetics of the individual astronauts (and cosmonauts). Therefore, improved human and animal models need to be developed for simulated weightlessness to better standardize and control the experimental conditions so that the cardiovascular and hormonal adaptations can be fully characterized.

We propose that rats infused with 20-25% excess blood from donor rats will mimic the increased cardiopulmonary volume seen in weightlessness and thus constitute a model for the study of fluid shifts and cardiovascular changes. We speculate that the hypervolemic/volume expansion model will not have the previously described deficiencies. Use of an animal model will allow blood collection and cardiovascular monitoring that is not feasible on human subjects. Direct comparisons can be made between these animal studies and those conducted during space flight. Furthermore, these ground support studies will provide valuable data to help differentiate and distinguish the fluid balance and control adaptation mechanisms from the bone and muscle changes observed in other animal models of weightlessness and during space flight.

In future studies, we will infuse compatible and physiologically comparable donor blood, as described and characterized above, into recipient rats, to simulate the fluid shifts of weightlessness. These experiments will describe the fluid balance factors (cardiovascular, renal and hormonal) operative in simulated weightlessness. In this manner, we will obtain a complete picture of the fluid shifts during simulated weightlessness and the attendant compensatory adjustments. These experiments may also provide data that can be used to develop protective measures for the debilitating effects of space flight and determine whether weightlessness will accelerate or ameliorate some cardiovascular diseases.

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