Hormonal and Electrolyte Responses to Acute Isohemic Volume Expansion in Unanesthetized Rats (43543)

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Abstract. **This study was undertaken to explore the time course of the metabolic response to isohemic blood volume expansion (30%) in normotensive, unanesthetized Sprague-Dawley rats. Whole blood, drawn from a femoral artery catheter of conscious donor rats, was infused into the jugular vein of recipient rats. Blood samples were drawn from a carotid artery of recipient rats at time points beginning immediately post-volume expansion (IPVE) up through 5 days post-volume expansion (PVE). To characterize the attendant compensatory mechanisms, the plasma concentrations of electrolytes and fluid regulatory hormones were determined. Hematocrit began to raise IPVE and was significantly elevated above control IPVE 20, 30, 40, 60, and 90 min, and 2, 4, 6, 8, 12, and 24 hr PVE. Consistent with our current understanding of the hormonal response to excess volume, atrial natriuretic factor was significantly increased above the prevolume expansion (control) values 0–30 min PVE. Surprisingly, plasma aldosterone levels were significantly increased above control at 20 and 30 min and 6 hr PVE, whereas plasma renin activity was significantly decreased 30–40 min PVE. Plasma sodium was not changed from control values except for a significant increase at 6 hr post-volume expansion. Plasma potassium, osmolality, and arginine vasopressin levels were not altered by the volume expansion. These studies delineate the physiologic time scheme operative in the regulation of fluid volume during acute isohemic volume expansion.**

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The renin-angiotensin system and the renal-vasopressin-thirst-aldosterone system have long been known to play an integral role in the control of body fluid composition. In the last 10 years, atrial natriuretic factor, a powerful blood volume and salt-regulating hormone, has been added to the scheme. The typical hormonal response to an expansion of blood volume includes a decrease in plasma arginine vasopressin (1), plasma renin activity (2), and aldosterone (3), whereas atrial natriuretic factor increases (4). Volume expansion with whole blood (5, 6), saline (7), and albumin (8) induces diuresis and natriuresis. There is a consequential decrease in plasma sodium with volume expansion using saline and albumin. No information is available on plasma electrolyte values for conscious isohemic volume-expanded rats.

The majority of the acute volume expansion studies have been confined to the study of one or two variables and a limited time scale. This is primarily due to the volume of blood that could be obtained from small experimental subjects without prompting physiologic changes that would interfere with the hypervolemic changes under investigation. Furthermore, the majority of investigators using animals as subjects have studied the response to volume expansion in anesthetized rats or dogs. Anesthesia has been shown to alter plasma hormone levels (1, 9). Comprehensive characterization of the compensatory adjustments in the fluid regulatory processes in acute volume expansion have not been reported.

The current study investigates the alterations in plasma electrolytes and volume-sensitive hormones seen in response to isohemic blood volume expansion.

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The model is one of unanesthetized donor-recipient infusion of whole blood with collection of blood samples from the carotid artery immediately after blood infusion up to and including 5 days post-volume expansion (PVE). Blood specimens were analyzed for hematocrit, plasma sodium, potassium, osmolality, arginine vasopressin (AVP), plasma renin activity (PRA), aldosterone (ALDO), and atrial natriuretic factor (ANF).

Materials and Methods

Experimental Design. Sprague-Dawley rats were obtained from Taconic Laboratories (Germantown, NY) and allowed to recover from the stress of transport under controlled temperature and humidity conditions for 2 to 4 days before surgery was performed. All rats were maintained on a 12:12-hr light:dark cycle, with the lighted phase beginning at 7 AM. Standard rat chow and water were available *ad libitum* throughout the experiment. The donor animals were cannulated when they reached a minimum body weight of 460–500 g. The recipient rats were cannulated when they were a minimum of 200 g but no more than 315 g.

The metabolic response to acute volume expansion was studied in conscious male Sprague-Dawley rats via an infusion of whole blood from matched, compatible, conscious donor rats representing an increase in blood volume for the recipient rat of 30%. Blood samples were drawn (immediately after volume expansion, up to and including 5 days after volume expansion) at specified time points for the measurement of plasma hormone and electrolytes.

Surgery. The rats were anesthetized using KETASET (75 mg/ml, ketamine hydrochloride; Aveco Co., Inc., Fort Dodge, IA) and GEMINI (10 mg/ml, xylazine; The Butler Co., Columbus, OH) injected (0.1 ml/100 g) intramuscularly. The donor rats were cannulated with a Teflon catheter (i.d. 0.022 in, o.d. 0.042 in; Cole-Parmer Instrument Co., Chicago, IL) inserted into the femoral artery of the right leg, anchored into place with Mersilene mesh (Carolina Surgical Supply Co., Raleigh, NC). The catheter was filled with 20% heparinized saline to maintain patency, plugged, and coiled in a protective nickel brass button on the animal's back. The recipient rats were cannulated with PE-50 catheters (i.d. 0.023 in, o.d. 0.038 in; Clay Adams/Becton Dickinson & Co., Parsippany, NJ) in the jugular vein and carotid artery. Each catheter was anchored with 5-0 silk suture, checked for patency, filled with heparinized saline, and heat sealed. All catheters were led subcutaneously under the skin to emerge from the back of the neck and coiled in a protective nickel brass button. Immediately after the surgeries, all rats received DI-TRIM (0.1 ml/100 g, 24%, trimethoprim sulfadiazim; Syntex Animal Health, West Des Moines, IA) subcutaneously as a prophylactic agent against infection. The rats were individually housed and allowed to recover 5–7 days after the surgery.

Protocol for Volume Expansion and Blood Sample Collection. At the end of the recovery period, the catheters of the donor and recipient rats were checked for patency and blood was obtained for hematocrit and a major side cross-match. No more than 250 μl of blood was obtained from the rats for these tests. No incompatibilities were detected.

All recipient rats received a volume of donor blood equal to 30% of their estimated control blood volume (estimated as 64 ml/kg body wt) (10). The blood for infusion was drawn as quickly as possible from the femoral artery of the donor rat using a heparinized syringe. A previous study demonstrated that up to 8 ml of blood drawn in less than 100 sec from the femoral artery of donor rats is comparable with the blood of the recipient rats and suitable for isohemic volume expansion studies (11). Measurement of levels of vasoactive and stress hormones (AVP, corticosterone, and catecholamines) showed that these were not elevated when the sample was obtained quickly. The blood was carefully mixed in the syringe and the air bubbles were removed. The blood was immediately infused into the jugular vein catheter of the recipient rat at a rate of 1.5 ml/min using a Harvard compact syringe pump (model 975; Harvard Apparatus, South Natick, MA). The venous catheter was flushed with heparinized saline and heat sealed at the end of the infusion. Nonspecific stressful stimuli were avoided and rats exhibiting behavioral agitation were excluded from the study (12, 13). The studies were performed between the hours of 9 AM and 3 PM.

The rats were randomly given a code designating the blood collection time. At the designated time, the heparinized saline solution filling the catheter was removed (approximately 0.25 ml) and the blood specimens were rapidly drawn in a clean, dry 3 ml syringe. The maximum volume of blood drawn at one time from a recipient rat for the hormone and electrolyte determinations was 3 ml. A sample (0.75 ml) was drawn at a time point soon after volume expansion (i.e., 10, 20, 30, or 40 min) and then 3 ml were drawn at one of the later time points (no less than 24 hr later). Approximately 100 μl were used for the hematocrit determination and the remaining portion of the specimen was added to the appropriate microsample tube for the hormone (ALDO, AVP, ANF, or PRA) assays. For the determination of ALDO and AVP, the blood was mixed with ammonium heparin (MICROTAINER tube with ammonium heparin; Becton Dickinson & Co., Rutherford, NJ). For the determination of PRA and ANF, the blood samples were mixed with EDTA (MICROTAINER tube with EDTA; Becton Dickinson). The blood was immediately centrifuged (900g) at 4°C. The plasma samples were removed from the red cells and

frozen in separate microcentrifuge tubes at −20°C until assayed.

Laboratory Procedures. Hematocrit was determined immediately after collection using the microhematocrit procedure (14). Plasma sodium and potassium concentrations were determined by flame photometry (15) and plasma osmolality by freezing point depression (osmometer model 110; Fiske Osmometer, Needham, MA) (16). AVP was measured by radioimmunoassay. Heparinized plasma samples (1 ml) were extracted with acetone and petroleum ether prior to radioimmunoassay, lyophilized, and stored frozen (−70°C) until assayed (17). The antiserum (final dilution of 1/180,000) was supplied by Dr. R. A. Eskay (NIAA, Bethesda, MD). The ^{125}I-labeled hormone was purchased from Dupont, Inc., Wilmington, DE. The 80% and 50% mean effective doses (ED_{80} and ED_{50}, respectively) were 1.21 and 4.31 pg/tube, respectively. ALDO was measured using 200 μl of heparinized plasma by a solid-phase radioimmunoassay that did not require extraction (DPC Coat-A-Count aldosterone procedure; Diagnostics Products Corp., Los Angeles, CA). The aldosterone procedure detects as little as 1.6 ng/dl. PRA was quantitated using 1.0 ml of EDTA plasma by the radioimmunoassay of generated angiotensin I (GammaCoat; Baxter Healthcare Corp., Cambridge, MA). The calculated sensitivity for PRA was 0.18 ng/tube. ANF was assayed by a double-antibody precipitation radioimmunoassay method (18, 19). Plasma samples (500 μl of EDTA plasma) were extracted using a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) activated with methanol and distilled water. The sample was applied and washed with 4% acetic acid followed by distilled water. ANF was eluted from the column with 75% acetonitrile and 25% acetic acid (4%). Atriopeptin III (Peninsula Laboratories, Belmont, CA) was the standard used. The antiserum (final dilution of 1/180,000) was made in rabbits against α-rat/(r) ANF (5–28)-coupled thyroglobulin (Dr. R. L. Eskay). The ^{125}I-labeled hormone was purchased from Dupont, Inc. The ED_{80} and ED_{50} were 8.10 and 36.38 pg/tube, respectively. The antisera were highly specific for each of the respective hormones, with little or no cross-reactivity to other compounds that might be present in the samples.

Statistical Analysis. Average values of ANF, PRA, ALDO, AVP, sodium, potassium, and osmolality after volume expansion were compared with baseline values using Student's *t* tests, with Bonferroni's adjustment of *P*-values to control the experiment-wise error rate (for each value) at $P = 0.05$ (20). Values at each time point were assumed to be independent of baseline values, although this assumption was not valid in all comparisons.

Results

Intravascular volume expansion by 30% with whole blood increased hematocrit from the control value of 44% to a peak value of 54.4% at 6 hr after volume expansion (Fig. 1).

ANF increased dramatically by 156% at 30 min PVE (Fig. 2). This increase was short-lived and ANF returned to control levels within 40 min PVE. ALDO, depicted in Figure 3, was significantly increased above control at 20 and 30 min and 6 hr PVE ($P \leq 0.01$). The ALDO values were not different from control values by 24 hr PVE. Using the limited amount of

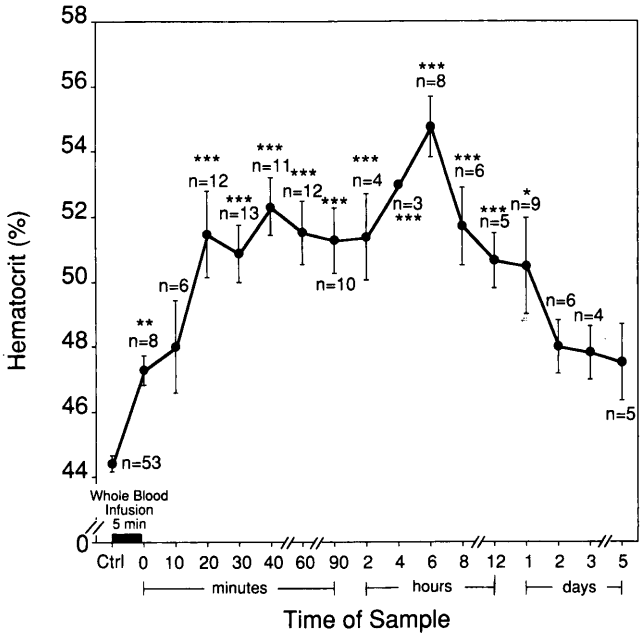

Figure 1. Response of hematocrit to 30% acute isohemic volume expansion. Values are mean ± SE. *$P \leq 0.05$; **$P \leq 0.001$; ***$P \leq 0.0001$.

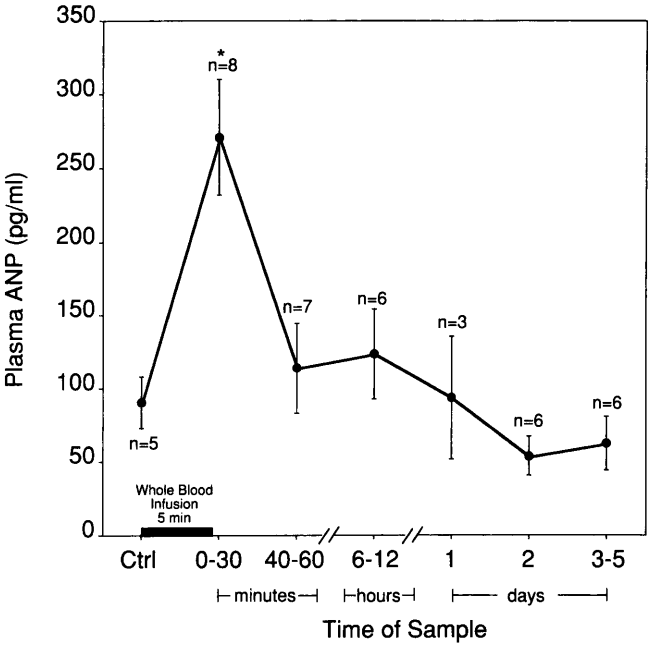

Figure 2. Response of plasma atrial natriuretic peptide to 30% acute isohemic volume expansion. Values are mean ± SE. *$P \leq 0.02$.

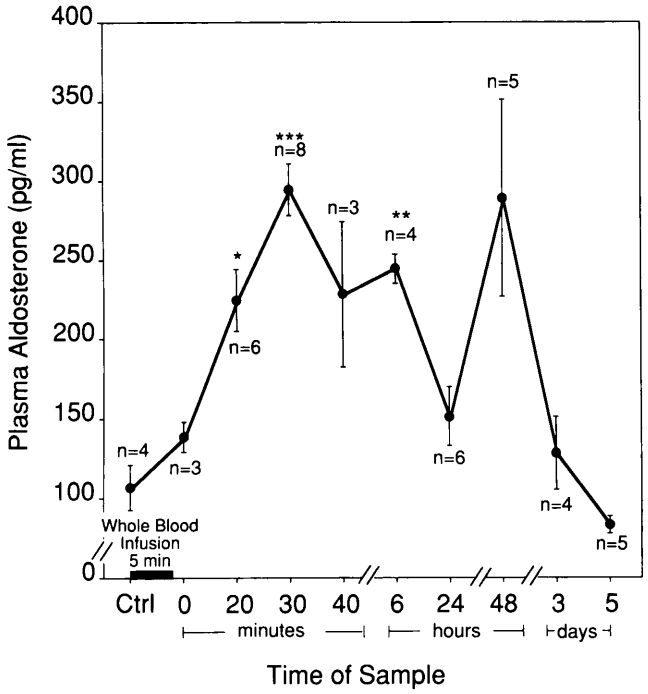

Figure 3. Response of plasma aldosterone to 30% acute isohemic volume expansion. Values are mean ± SE. *$P \leq 0.01$; **$P \leq 0.001$; ***$P \leq 0.0001$.

plasma remaining from the ANP determinations, PRA levels were determined on three groups of rats (control, 0–30 min, and 3–5 days PVE). PRA control values were 30.2 ± 2.85 ng/ml ($n = 3$) and PRA values 0–30 min PVE (12.0 ± 0.88 ng/ml, $n = 7$) were significantly decreased below control levels ($P \leq 0.05$). The PRA levels were not different from control levels at 3 days PVE.

Plasma sodium, potassium, and OSM were not significantly different from the control values at any of the time points after volume expansion (Fig. 4), with the exception of sodium at 6 hr PVE ($P \leq 0.01$).

AVP, depicted in Figure 5, was not significantly changed at any time point after volume expansion.

Discussion

Complete characterization of fluid control processes from the onset of an imbalance (such as hypervolemia) and an integrated understanding of the operative metabolic pathways in unanesthetized animals have not been possible to date.

The use of anesthesia in most of the early studies is a problem, since anesthesia itself can cause an aberration of the adaptation processes, thereby making these observations difficult to interpret. Plasma hormone levels are affected by anesthetics (1, 9) and the method of sample collection (21). One must also consider the time course of the changes in response to volume expansion. Although fluid exchange processes between the intravascular and extravascular portions of the extracellular fluid space begin to occur in minutes (22), interstitial pressure requires hours (23) and total body sodium

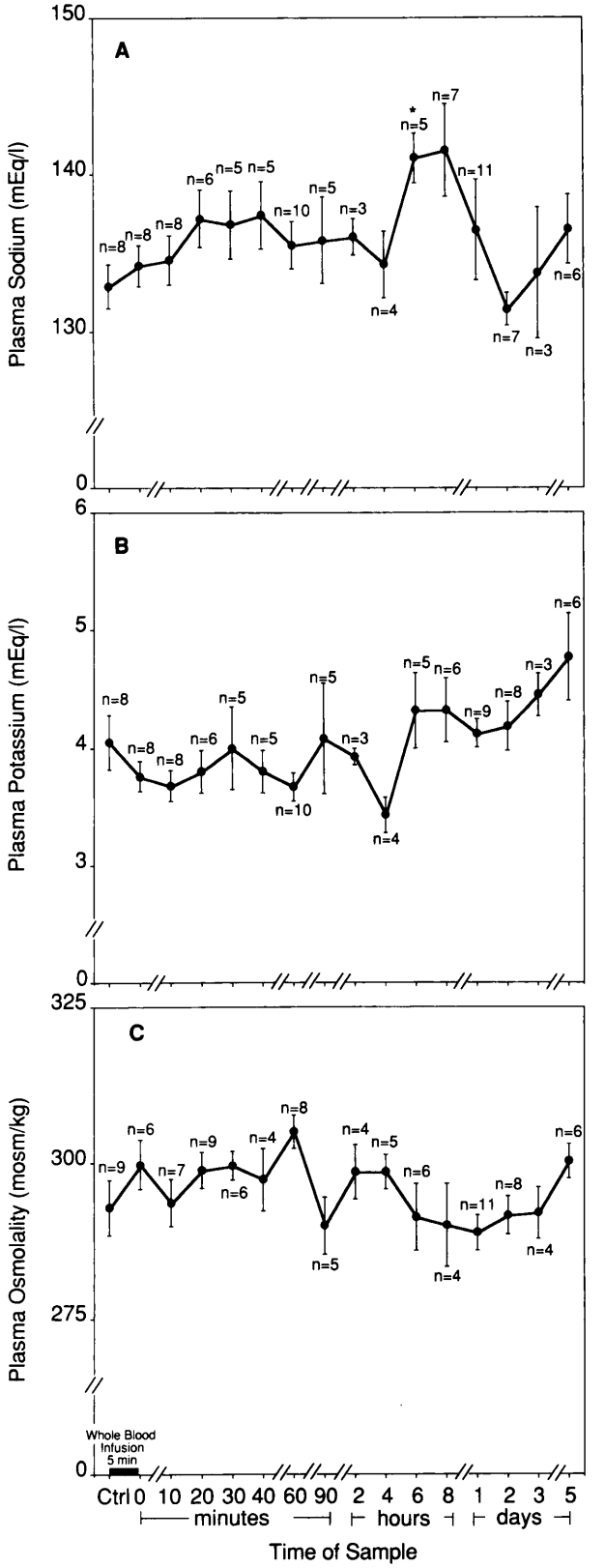

Figure 4. Responses of (A) sodium, (B) potassium, and (C) osmolality to 30% acute isohemic volume expansion. Values are mean ± SE. *$P \leq 0.01$.

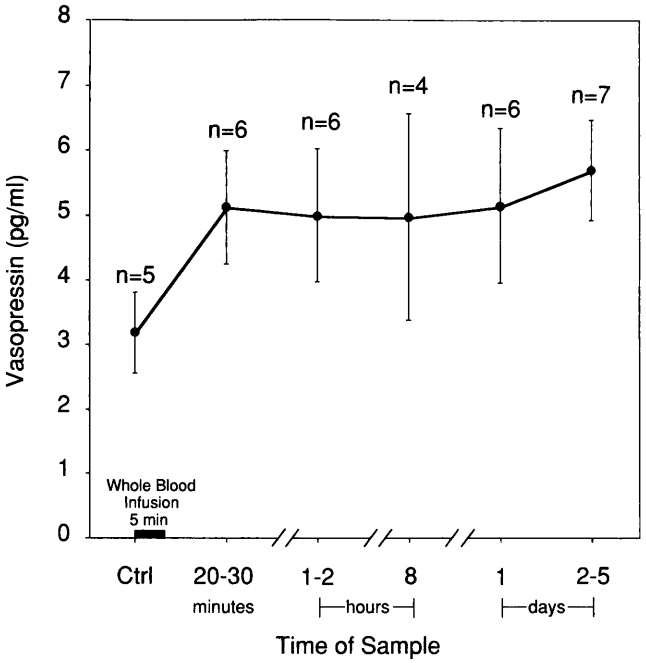

Figure 5. Response of plasma arginine vasopressin to 30% acute isohemic volume expansion. Values are mean ± SE.

regulation may require days for complete homeostasis (22).

In the current studies, volume expansion using whole blood caused a significant increase in hematocrit. There was a 22% increase at 20 min and the hematocrit remained elevated until 24 hr PVE. This is consistent with the results of other volume expansion studies in rats utilizing whole blood (24). This hemoconcentration is probably the result of renal excretion of sodium and water and transcapillary extracellular fluid flux.

ANF has been shown to be a potent diuretic, natriuretic, and vasodilator that increases immediately after blood volume expansion with saline or blood (4, 19, 25, 26). Many of the studies conducted to characterize the response of ANF to acute volume expansion have been confounded by the use of anesthetics that elevate the basal ANF levels (4, 7, 13). Volume expansion studies in intact conscious rats using whole blood demonstrated a 265% increase in plasma ANF 5 min after a 20% volume expansion (26). The current studies support these findings in that there was a significant increase in ANF at 30 min PVE.

In agreement with the currently held hypotheses regarding the mechanisms controlling renin release, PRA was significantly decreased by volume expansion. Studies in which central blood volume expansion is induced by head-down tilt or water immersion have reported decreased PRA (27). The renin-angiotensin system has been shown to stimulate aldosterone secretion from the adrenal cortex (2). Thus, the paradox in the present studies is that, despite a significantly decreased PRA, ALDO was increased.

Aldosterone's role in the control of blood volume is to modify sodium and potassium homeostasis (28), thereby increasing the excretion of potassium and hydrogen ions, raising arterial pressure, and decreasing renin release (29). Contrary to our current understanding of the mechanisms controlling plasma aldosterone secretion, when blood volume was increased in our rats, ALDO secretion was stimulated. Interestingly, increased plasma aldosterone levels have been observed in head-down suspension studies in rats (30) and bed rest studies using human subjects (27, 31). These investigators have no explanation for these divergent findings.

A number of factors have been shown to stimulate aldosterone secretion, including the angiotensins (II and III), renin, potassium, ACTH, and neurotransmitters. ANP is the only factor known to inhibit aldosterone secretion (32). Himathongkam *et al.*'s (33) study suggests that plasma aldosterone is sensitive to small changes in plasma potassium concentrations within the physiologic range. A significant increase in ALDO was produced by a 0.1- to 0.5-mEq/liter potassium infusion without a statistically significant rise in plasma potassium levels (33). Small changes in plasma potassium could not be detected in the current studies. Increased biosynthesis of aldosterone has also been associated with increased adrenocortical potassium that may be mediated by ACTH (33). Furthermore, ACTH has been shown to stimulate aldosterone secretion (33). Although, the effect of ACTH on aldosterone stimulation is reputedly short lived, the aldosterone response may be modulated synergistically by intracellular and extracellular electrolytes (sodium and/or potassium) or other hormones (cortisol, renin, and ANF) (29, 33, 34). Dissociation of the renin-angiotensin-aldosterone axis has been reported in other studies (35) and observed in space flight (36) and thus warrants further investigation.

Saline volume expansion studies in dogs (1), water immersion studies (27, 37), and some head-down tilt studies in humans (38) have demonstrated decreased AVP in response to volume expansion. Other studies have shown little or no change in AVP after volume expansion (39, 40). Morris *et al.* (41) speculated that AVP responds more quickly and dramatically to hemorrhage, whereas ANF is more responsive to volume expansion. The lack of change in AVP observed in the current studies may be due to a number of factors, including the basal hydration level and dietary sodium intake, the expansion solution, and the rate of the expansion. The use of solutions for expansion other than whole blood results in hemodilution, and hemodilution may be the stimulus for the inhibition of AVP release (42).

The findings observed in the current studies are evidence of the complexity of the interactions among and between the renin-angiotensin-aldosterone axis, the renal-arginine vasopressin system, and ANF, and serve to characterize the volume regulatory hormone and

plasma electrolyte response to acute isohemic volume expansion in the conscious animal.

The response of the fluid regulatory hormones (ALDO, AVP, PRA, and ANF) to 30% acute isohemic volume expansion corresponds, in part, to the conventional theories regarding control of blood volume. However, ALDO was increased and AVP was not changed by volume expansion. The responses observed in the current studies may reflect a dissociation of the renin-angiotensin-aldosterone system and the renal-AVP system. The changes may also be explained by the interaction of ANF and plasma electrolytes with the other volume regulatory hormones (AVP, ALDO, and PRA). Additional studies are needed to delineate more thoroughly the complete time scheme in hypervolemia and elucidate the attendant compensatory adjustments, incorporating fluid consumption and the thirst mechanism, intracellular electrolyte levels, and urine excretion.

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1. Cowley AW Jr. Vasopressin and cardiovascular regulation. Int Rev Phys **26**:189–242, 1982.
2. Kimura T, Minai K, Matsui K, Mouri T, Sato T, Yoshinaga K, Hoshi T. Effects of various states of hydration on plasma ADH and renin in man. J Clin Endocrinol Metab **42**:79–87, 1976.
3. Williams GH, Tuck ML, Rose LI, Dhuly RG, Underwood RH. Studies of the control of plasma aldosterone concentration in normal man. J Clin Invest **5**:2645–2652, 1972.
4. Buckalew VM Jr, Paschal-McCormick C. Natriuretic hormone: Current status. Am J Nephrol **9**:329–342, 1989.
5. Hirth C, Stasch J-P, John A, Karzda S, Morich F, Neuser D, Wohlfeil S. The renal response to acute hypervolemia is caused by atrial natriuretic peptides. J Cardiovasc Pharmacol **8**:268–275, 1986.
6. Garcia R, Cantin M, Thibault G. Role of right and left atria in natriuresis and atrial natriuretic factor release during blood volume changes in the conscious rat. Circ Res **61**:99–106, 1987.
7. Morris M, Alexander N. Baroreceptor influences on plasma atrial natriuretic peptide (ANP): Sinoaortic denervation reduces basal levels and the response to an osmotic challenge. Endocrinology **122**:373–375, 1988.
8. Ackermann U. On the regulation of the renal response to blood volume expansion by vascular parameters in the rat. Pflugers Arch **355**:151–164, 1975.
9. Chiu PJS, Vemulapalli S, Policelli M, Kifor I, Sybertz EJ, Dzau VJ. The responses of atrial natriuretic factor concentrations to acute volume changes in conscious rats. Life Sci **41**:2339–2347, 1987.
10. Trippodo NC, Yamamoto J, Frolich ED. Whole-body venous capacity and effective total tissue compliance in SHR. Hypertension **3**:104–112, 1981.
11. Chenault VM, Lynch CD, Morris M, Clodfelter J, Hutchins PM. Characterization of blood drawn rapidly for the use in blood volume expansion studies: An animal model for simulated weightlessness. VISION-21 Symposium Conference Proceedings. Washington, DC: U.S. Government Printing Office, CP-10059, 1991.
12. Popper CW, Chineh CC, Kopin IJ. Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization and after decapitation. J Pharmacol Exp Ther **202**:144–148, 1977.
13. Blizard DA, Morris M. Acute stress increases plasma concentrations of atrial peptides. Proc Soc Exp Biol Med **184**:123–126, 1987.
14. Fox KR, Parker DM, Crass G. Hematological evaluation: Peripheral blood in laboratory medicine. Race GJ, Ed. Philadelphia: Harper & Row, pp1–60, 1965.
15. Weisman N, Pileggi VJ. Inorganic ions. In: Henry RJ, Cannon DC, Winkleman JW, Eds. Clinical Chemistry- Principles and Techniques, 2nd ed. Hagerstown, MD: Harper & Row, pp639–754, 1974.
16. Anonymous. The physical chemistry, theory and technique of freezing point determinations. Needham, MA: Advanced Instruments, Inc., 1971.
17. Alexander N, Morris M. Effects of chronic sinoaortic denervation on central vasopressin and catecholamine system. Am J Physiol **255**:R768–R773, 1988.
18. Morris M. Neurohypophyseal response to dehydration in the spontaneously hypertensive rat. Hypertension **4**:161–166, 1982.
19. Rauch AL, Callahan MF, Buckalew VM Jr, Morris M. Regulation of plasma atrial natriuretic peptide by the central nervous system. Am J Physiol **258**:R531–R535, 1990.
20. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. Circ Res **47**:1–9, 1980.
21. Oates HF, Stokes GS. Renin stimulation caused by blood collection techniques in the rat. Clin Exp Pharmacol Physiol **1**:495–501, 1974.
22. Hinghofer-Szalkay H. Fluid volume changes in astronauts—How they fit our understanding of physiology. Proceedings of the third European Symposium on Life Sciences Research in Space. Graz, Austria, September 14–18, 1987. European Space Agency Special Publication **271**:135–142, December 1987.
23. Ackermann U. Changes in interstitial pressure during and after blood volume expansion in rats. Pflugers Arch **374**:291–296, 1976.
24. Ackermann U. Cardiac output and renal excretion rates during acute blood volume expansion in rats. Am J Physiol **234**:H21–H27, 1978.
25. Lang RE, Thoken H, Ganten D, Luft FC, Ruskoaho H, Unger TH. Atrial natriuretic factor—A circulating hormone stimulated by volume loading. Nature **314**:264–266, 1985.
26. Pettersson A, Hedner J, Ricksten S-E, Towle AC, Hedner J. Acute volume expansion as a physiological stimulus for the release of atrial natriuretic peptides in the rat. Life Sci **38**:1127–1133, 1986.
27. Gharib C, Gauquelin G, Geelen G, Cantin M, Gutskovska J, Mauroux JL, Guell A. Volume regulating hormones (renin, aldosterone, vasopressin and natriuretic factor) during simulated weightlessness. Physiologist **28**:S30–S33, 1985.
28. Gibbons GH, Dzau VJ, Farhi ER, Barger AC. Interaction of signals influencing renin release. Annu Rev Physiol **46**:291–308, 1984.
29. Laragh JH. Atrial natriuretic hormone, the renin-aldosterone axis and blood pressure-electrolyte homeostasis. N Engl J Med **313**:1330–1340, 1985.
30. Musacchia XJ, Steffen JM. Cardiovascular and hormonal (aldosterone) responses in a rat model which mimics responses to weightlessness. Physiologist **27**:S41–S42, 1984.
31. Volicer L, Jean-Charles R, Chobanian AV. Effects of head-down tilt on fluid and electrolyte balance. Aviat Space Environ Med **47**:1065–1068, 1976.

32. Quinn SJ, Williams CJ. Regulation of aldosterone secretion. Annu Rev Physiol **50**:409–426, 1988.
33. Himathongkam T, Dluhy RG, Williams GH. Potassium-aldosterone-renin interrelationships. J Clin Endocrinol Metab **411**:153–159, 1975.
34. Baumer JS, Davis JO, Johnson JA, Twitty RT. Increased adrenocortical potassium in association with increased biosynthesis of aldosterone. Am J Physiol **220**:1094–1099, 1971.
35. Findling JW, Waters VO, Raff H. The dissociation of renin and aldosterone during critical illness. J Clin Endocrinol Metab **64**:592, 1987.
36. Frey MAB, Charles JB, Houston DE. Weightlessness and response to orthostatic stress. In: Smith JJ, Ed. Circulatory Response to the Upright Posture. Boca Raton, FL: CRC Press, pp65–120, 1990.
37. Epstein M, Preston S, Weitzman RE. Isosmotic central blood volume expansion suppresses plasma arginine vasopressin in normal man. J Clin Endocrinol Metab **52**:256–262, 1981.
38. Nixon JV, Murrary RJ, Bryant C, Johnson RJ, Mitchell JH, Holland OB, Gomez-Sanchez C, Vergne-Marini P, Blomqvist CG. Early cardiovascular adaptation to simulated zero gravity. J Appl Physiol **46**:541–548, 1979.
39. Chobian AV, Lille DL, Tercyak A, Belvins P. The metabolic and hormonal effects of prolonged bedrest in normal subjects. Circulation **49**:551–559, 1974.
40. Kravik SE, Keil LC, Silver JE, Wong N, Spaul WA, Greenleaf JE. Immersion diuresis without expected suppression of vasopressin. J Appl Physiol **57**:123–128, 1984.
41. Morris M, Cain M, Chalmers J. Complementary changes in plasma atrial natriuretic peptide and antidiuretic hormone concentrations in response to volume expansion and hemorrhage studies in conscious normotensive and spontaneously hypertensive rats. Clin Exp Pharmacol Physiol **14**:283–289, 1987.
42. Gilmore JP, Zucker IH, Ellington MJ, Richards MA, Share L. Failure of acute intravascular volume expansion to alter plasma vasopressin in the nonhuman primate, Macaca fascicularis. Endocrinology **106**:979–982, 1980.