Renal responsiveness to aldosterone during exposure to simulated microgravity

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METHODS

Subjects. Six mature male rhesus monkeys (Macaca mulatta) averaging 7.6 ± 0.3 kg (range 7.0–8.6 kg) in weight were selected as subjects for this study. All experimental procedures and protocols were reviewed and approved by the

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THE KIDNEYS CONTRIBUTE significantly to the control of vascular volume. A 10–20% reduction in plasma volume is one of the fundamental adaptations to a reduced influence of gravity, such as head-down tilt (HDT) bed rest and space flight (1). Lower vascular volume induced in ground-based models of microgravity has been associated with acute diuresis and natriuresis (1, 5, 6, 17). Normal baseline plasma renin activity (2 ng ANG II·ml−1·h−1) and plasma aldosterone (130–145 pg/ml) have been elevated to as much as 6 ng ANG II·ml−1·h−1 and 225 pg/ml, respectively, in healthy human subjects after exposure for 16–21 days of HDT (2, 7) and spaceflight (16), whereas renal Na+ excretion has been either unaltered or increased (2, 7, 16). Attempts to restore plasma volume with isotonic fluid drinking or infusion in human subjects exposed to HDT have failed (5, 19). Taken together, these observations may be explained in part by less sensitivity of renal distal tubular cells to aldosterone after exposure to HDT, with a subsequent reduction in renal capacity for Na+ retention.

We hypothesized that elevated Na+ and water excretion observed during prolonged exposure to microgravity and the subsequent inability to restore body fluids by drinking or saline infusion might be reflected, at least in part, by reduced renal tubular responsiveness to aldosterone. If renal tubular responsiveness to aldosterone were reduced with exposure to microgravity, then we would expect measures of renal Na+ retention to be less when a bolus of aldosterone was administered during HDT compared with a control experimental condition. To test this hypothesis, we conducted an investigation in which we administered an acute bolus of aldosterone (stimulus) and measured responses in renal functions that included renal clearances of Na+, total renal Na+ excretion, and fractional Na+ excretion observed during prolonged exposure to microgravity. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Institutional Animal Care and Use Committee. The monkeys received 2 months of tilt-table adaptation training before the experiments. This training involved three phases consisting of 1) preliminary caretaker handling, restraint jacket fitting, and light ketamine sedation; 2) acute restraint jacket and tilt-table acclimation training (<2 h); and 3) increased restraint jacket and tilt-table adaptation training (up to 24 h).

After verification that monkeys were able to adapt to the tilt table during all phases of training, they were chronically instrumented with an indwelling jugular catheter that was advanced to terminate in the anterior vena cava just outside the right atrium. This catheter provided an access site for acute insertion of a single-tip 3-Fr micromanometer (Millar Instruments, Houston, TX) for withdrawal of serial blood samples and administration of exogenous aldosterone. Subjects were given at least 1 wk of postoperative recovery before the start of the test protocol.

Experimental design. A standard two-treatment crossover design was used, with each monkey receiving both 10° HDT and upright/prone control conditions. The use of the rhesus monkey placed in the 10° HDT position was chosen because actual changes in cardiovascular and renal responses reported in humans during exposure to actual spaceflight have been closely simulated by this ground-based animal model (1, 3, 9). The treatment order was randomized but counterbalanced, so that three monkeys received HDT followed by the control condition and three monkeys received the control condition followed by HDT. Each treatment period lasted 96 h (4 days). The monkeys were kept unrestrained in their cages for a period of 9 days between treatment periods (i.e., crossover interval). The temperature and humidity of the laboratory and cage rooms were maintained at 24 ± 1°C and ~40% relative humidity. An additional “time” (repeated-measures) effect independent of the posture treatments was introduced because some of the dependent variables under study were measured over various time courses.

Measurement techniques. Test subjects were trained and tested on custom designed and fabricated HDT tables that were positioned at one of three settings, as previously described (9): 10° HDT, 0° prone, and 80° head-up tilt. Animals were continuously monitored throughout the experimental procedures. The control condition consisted of 16 h of 80° head-up tilt (0700–2300) and 8 h of 0° prone to provide a sleeping posture (2300–0700). The HDT treatment condition consisted of continuous exposure (i.e., 24 h/day) in 10° HDT. During each experimental condition, water and food were provided ad libitum, and amounts of intake were recorded. A standard monkey diet biscuit (LabDiet) of ~3.5 g with caloric density equal to 4 kcal/g (69% carbohydrate, 13% fat, 18% protein) was used as the primary food source. The monkeys were kept unrestrained in their cages for a period of 9 days between treatment periods.

Aldosterone infusion test. From ~0800 to 1300 on day 4, each animal was lightly sedated by use of a steady-state infusion of ketamine (0.15 mg·kg⁻¹·min⁻¹) and placed in the prone (0°) posture for the aldosterone infusion experiment. We chose to standardize the position of the animal in the prone posture during the aldosterone administration test in both control and HDT conditions so that we could eliminate posture as a contributing factor to physiological responses. The initial hour of the protocol was used to place a temporary catheter in the saphenous vein and to insert a 5-French Foley urinary catheter for collection and measurement of urine volume. At ~0900, each animal received a bolus infusion of aldosterone (1 mg iv) added to a baseline maintenance infusionate (0.4 ml·kg⁻¹·min⁻¹ 0.9% saline) through the saphenous vein catheter to assure adequate hydration state throughout the 4-h experiment. Infusions of both ketamine and aldosterone bolus were given via the atrial access catheter. The aldosterone infusion dose was determined during preliminary experiments conducted in our laboratory on four monkeys that demonstrated its ability to significantly increase plasma aldosterone concentrations and reduce urine Na+/K+ ratio (Fig. 1). The subjects’ bladders were evacuated every 30 min throughout the experimental period. Blood samples (2 ml each) were taken each 60 min during the experimental period to measure plasma concentrations of Na⁺, K⁺, osmolality, and creatinine and to calculate renal clearances of these solutes. The blood samples withdrawn at baseline and each 60 min during the experimental period were also used to calculate percent change in plasma volume from changes in venous hematocrit (18). An additional volume of blood (6 ml each) was withdrawn at 0, 60, 120, and 240 min after aldosterone administration for determination of plasma renin activity, aldosterone, arginine vasopressin (AVP), and atrial natriuretic peptide (ANP). In addition, heart rate (electrocardiogram) and systolic, diastolic, and mean arterial blood pressures (noninvasive automated sphygmomanometry from the right arm) were measured every 30 min.

Responses in renal functions. Plasma and urine samples were analyzed for concentrations of Na⁺, K⁺, and creatinine by use of an ion-sensitive electrode system (Nova 16). Plasma and urine osmolality was measured by freezing-point depression (Advanced Instruments Model 3D3 osmometer). Urine flow rate (UVR) was calculated as the volume of urine collected divided by the time duration of the collection. Na⁺ excretion rate was calculated as urine Na⁺ concentration times UVR. Na⁺ and osmotic clearances were calculated as the rate of Na⁺ or osmotic excretion divided by the mean plasma concentrations. Free water clearance was equal to UVR – osmotic clearance. Glomerular filtration rate was estimated from the clearance rate of creatinine (creatinine excretion rate divided by plasma creatinine concentration). The filtered load for Na⁺ was calculated as the Na⁺ plasma concentration times glomerular filtration rate, and the percentage of the filtered load excreted for Na⁺ was calculated as the rate of excretion times 100 divided by the filtered Na⁺ load (20). The log(Na⁺ × 10/K⁺) ratio from measurements of Na⁺ and K⁺ concentrations in the urine was used to assess aldosterone effects (8).

Plasma hormone analysis. Radioimmunoassay (RIA) procedures were used to analyze plasma samples for aldosterone (Diagnostic Products, Los Angeles, CA), AVP (Phoenix Phar...
maceuticals, Mountain View, CA), α-ANP (Phoenix Pharmedaceuticals), and plasma renin activity (NEN Life Science Products, Boston, MA). For the determination of AVP and ANP, samples were extracted by using octadecyl (C-18) extraction columns (E & K Scientific, Campbell, CA) and were then assayed by RIA. Spiked recovery was 92%, sensitivity was 0.5 pg/ml, within-assay coefficient of variability (CV) was 2.8%, and between-assay CV was 9.9%. Measurement of plasma renin activity was performed by quantifying the amount of ANG I that was generated on the addition of substrate. The RIA was performed in the presence of reagents that inhibited endogenous angiotensin-converting enzyme and proteolytic angiotensinases. Spiked recovery was 96%, sensitivity was 0.1 ng·ml⁻¹·h⁻¹, within-assay CV was 2.7%, and between-assay CV was 5.5%. For determination of aldosterone, no extraction was used. Recovery was 93%, sensitivity was 15 pg/ml, within-assay CV was 7.3%, and between-assay CV was 8.3%. A standard was added to the lower end of the curve. The concentration of this standard was 50% of the lowest standard provided in the kit. Samples that were high and off the curve were diluted 1:8. In addition, parallelism tests demonstrated the measurement capabilities of the assay at higher aldosterone concentrations.

Statistical methods. A standard two-treatment (Control, HDT) by time within-subjects repeated-measures ANOVA was used to evaluate the main effects of treatment, time, and their subsequent interaction. Dependent variables included measures of renal function, plasma hormones associated with renal function, and hemodynamics. Statistical models for hemodynamic measures and plasma hormone levels differed from the renal function model only in the number of time periods observed. Exact P values were calculated for each independent effect and reflect the probability of observing the measured effect or one larger given only random error. Separate error terms were generated for each effect in the statistical model. Error bars and standard errors presented in figures and tables reflect simple standard errors around means and do not reflect variation specific to the experimental design or the variability associated with statistical tests.

RESULTS

Dietary intake. Average daily (24 h) fluid intake during HDT (400 ± 137 ml) was not statistically different (t = 0.58, P = 0.595) from that of the control condition (421 ± 171 ml). Daily calorie and Na⁺ intakes were 126 ± 19 kcal and 105 ± 17 mg in the control condition compared with 152 ± 25 kcal and 107 ± 22 mg during HDT (t ≥ 0.566, P ≥ 0.596).

Changes in baseline renal responses to HDT. Table 1 presents the mean ± SE values for measures of renal function by treatment condition and time period and includes summaries of the statistical tests of main effects. UVR was slightly reduced from 51 ± 3 ml/h in the control condition to 42 ± 3 ml/h in the HDT condition. Filtered Na⁺ load, renal clearance of creatinine, osmotic clearance, urinary Na⁺/K⁺ ratio, plasma osmolality, and plasma Na⁺ remained relatively unchanged by HDT (P ≥ 0.266). Na⁺ clearance and Na⁺ excretion increased with HDT (P ≤ 0.055), whereas renal clearance of water was reduced by an average change of −19 ml/h. Urine Na⁺ concentration and renal fractional excretion of Na⁺ also increased due to HDT (P = 0.020 and 0.069, respectively).

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<th>Table 1. Renal functions at baseline and during 4 h after administration of aldosterone in the control and HDT treatment conditions</th>
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Values are means ± SE. HDT, head-down tilt; Ccr, renal clearance of creatinine; CNa⁺, renal clearance of sodium; CTO₂, osmotic clearance; CNa⁺/K⁺, renal clearance of water; [Na⁺], sodium concentration. All statistical tests were based on 6 animals and were performed with 1 and 5 degrees of freedom. *Time (aldosterone) main effect; †treatment (HDT) main effect.

Changes in renal responses to aldosterone administration. Overall, UVR was unaffected by aldosterone infusion. Aldosterone administration did not alter filtered Na⁺ load, renal clearance of creatinine, plasma osmolality, or plasma Na⁺ (P ≥ 0.20). Na⁺ clearance, Na⁺ excretion, and Na⁺/K⁺ ratio decreased with aldosterone infusion (P ≤ 0.011). Although reductions in Na⁺ clearance and Na⁺ excretion due to aldosterone were greater during HDT than during control [P(1,5) ≥ 4.41, P ≤ 0.090], the differential (i.e., interaction) effect was minimal and tended to zero when changes in aldosterone were corrected for preinfusion levels. Osmotic clearance was reduced (18.4 ml/h) by aldosterone infusion, whereas renal clearance of water was increased by an average change of −19 ml/h. Urine Na⁺ concentration and renal fractional excretion of Na⁺ also increased due to HDT.
demonstrated indistinguishable changes due to aldosterone infusion ($P = 0.253$ and $0.152$, respectively). None of the aldosterone effects (interactions) on renal functions were altered by HDT.

**Hemodynamic responses.** Mean heart rate, arterial blood pressures, and relative (percent) change in plasma volume by treatment condition and time after aldosterone administration are presented in Fig. 2. Although plasma volume tended to increase after aldosterone infusion, neither heart rate, blood pressures, nor change in plasma volume showed statistically discernable effects of treatment [$F(1,5) = 1.897, P \geq 0.227$], time [$F(4,20) = 2.108, P \geq 0.118$], or their subsequent interaction [$F(4,20) = 0.833, P \geq 0.520$]. During the 4-h period after aldosterone administration, the animals increased ($P < 0.05$) their body weight by a similar amount in HDT ($167 \pm 65 \text{ g}$) and the control condition ($125 \pm 36 \text{ g}$).

**Hormone responses.** Mean plasma concentrations of aldosterone, plasma renin activity, vasopressin, and ANP by treatment condition and time after aldosterone administration are presented in Fig. 3. A large main effect of time was seen for aldosterone [$F(2,10) = 94.07, P = 0.0001$] as a result of aldosterone administration. Plasma aldosterone spiked immediately after infusion and then returned to baseline levels. Plasma renin activity and plasma vasopressin were unaffected by HDT treatment [$F(1,5) = 0.713, P = 0.437$] or time [$F(3,15) = 0.660, P \geq 0.589$], with no subsequent interaction effects [$F(3,15) = 1.489, P \geq 0.258$]. A large main effect of treatment condition was observed for ANP [$F(1,5) = 19.89, P = 0.0066$], with lower values resulting from HDT.

**DISCUSSION**

Although numerous investigators have reported the effects of exposure to actual or simulated microgravity on renal function (2, 4–7, 10–12, 14–17, 20) and volume-regulating hormones (2–4, 7, 10–12, 14–17), we are unaware of any previous study in which the contribution of aldosterone to the mechanism of microgravity-induced renal Na$^+$ excretion was directly examined with administration of aldosterone. In the present experiment, we administered aldosterone and compared responses of renal functions during HDT and a control upright posture in rhesus monkeys to test the hypothesis that elevated Na$^+$ excretion during exposure to microgravity might be reflected, at least in part, by reduced renal tubular responsiveness to aldosterone. We substantiated an elevation of Na$^+$ excretion during exposure to simulated microgravity by demonstrating increases in renal Na$^+$ clearance, renal Na$^+$ excretion, urine Na$^+$ concentration, and fractional Na$^+$ excretion during HDT compared with the control condition. However, we unexpectedly observed similar reductions in renal Na$^+$ and osmotic clearances, urine Na$^+$/K$^+$ ratio, renal Na$^+$ excretion, urine Na$^+$ concentration, and fractional Na$^+$ excretion during aldosterone administration in HDT compared with the control condition. Therefore, contrary to our hypothesis, renal
responsiveness to aldosterone was not altered during exposure to simulated microgravity. Our results suggest that reduction in renal Na\textsuperscript{+} retention during exposure to microgravity must be explained by a natriuretic mechanism(s) other than aldosterone.

**Responses to HDT.** We observed no alterations in arterial blood pressures or plasma Na\textsuperscript{+} concentration and osmolality during the aldosterone administration experiments, suggesting that there were no significant differences in Starling forces across the glomerular membrane between HDT and the control condition. Although direct assessment of renal blood flow has not been reported during actual spaceflight and was not measured in the present study, previous ground-based experiments indicated that it is not altered by bed rest (12). Like previous ground-based and spaceflight data (2, 4, 10–12), creatinine clearance values were virtually unchanged by HDT in the present study, suggesting that glomerular filtration rate was also unaltered by microgravity. The notion that glomerular filtration rate was not altered in our experiment was also supported by the absence of change in filtered Na\textsuperscript{+} load between HDT and control conditions. Taken together, these observations support the notion that increased Na\textsuperscript{+} excretion induced by exposure to simulated microgravity in our animals probably involved alterations in postglomerular mechanisms.

Reduced renal tubular retention of Na\textsuperscript{+} during exposure to microgravity may be affected by alterations in circulating plasma renin activity, aldosterone, ANP, or AVP or their interactions with renal tubular mechanisms. We observed no differences in plasma renin activity, aldosterone, or AVP between the HDT and control experiments. If circulating ANP contributed to elevated Na\textsuperscript{+} excretion during exposure to microgravity, we would have expected this factor to be elevated during HDT in our animals. However, similar to a previous observation in humans (2), ANP was reduced in the HDT condition compared with the control in the present study. Therefore, our results suggest that alteration in renal tubular receptor responsiveness represents a more likely explanation than circulating fluid-regulating hormones for reduced renal tubular retention of Na\textsuperscript{+} during exposure to microgravity. Specifically, our data raise the possibility that renal tubular responsiveness to ANP may have increased with exposure to microgravity.

Baseline plasma AVP, the antidiuretic hormone, was not altered by HDT in our monkeys. This finding was consistent with the observations that AVP was not altered in primates during water immersion (13), in humans during exposure to HDT bed rest (2), and in astronauts during spaceflight compared with their ground-based supine posture (16). We also observed reduced free water clearance during HDT compared

Fig. 3. Responses of plasma aldosterone, renin activity, vasopressin, and atrial natriuretic peptide at baseline and during 4 h after intravenous administration of aldosterone during HDT (○, dashed lines) and the control treatment (●, solid lines). Values are means ± SE.
with the control condition, a finding similar to that in humans (2). The relationship between enhanced renal tubular water absorption without change in plasma AVP suggests that renal distal tubular responsiveness to antidiuretic hormone may be increased by exposure to simulated microgravity.

We acknowledge that our results may not reflect renal adaptations that may occur during exposure to microgravity for more than 3–4 days. However, Haruna and co-workers (7) reported elevations in average plasma aldosterone and renal Na\(^+\) excretion at 3 days of HDT in 18 human subjects that were maintained at days 10 and 20 of HDT. Although we cannot dismiss the possibility that there might be altered renal responsiveness during adaptation to microgravity exposure beyond 3 days, the data of Haruna et al. suggest that our model for the study of the interrelationship between aldosterone and renal Na\(^+\) excretion after only 3 days can be maintained for as long as 3 wk.

**Responses to aldosterone infusion.** Normal or elevated Na\(^+\) excretion has been demonstrated during HDT despite elevated plasma renin activity and aldosterone (2, 7), suggesting the possibility of decreased renal tubular sensitivity to aldosterone. Under the experimental conditions of the present investigation, our observation that plasma renin activity and aldosterone were unaltered by HDT with elevated renal Na\(^+\) excretion was also consistent with the possibility that the renal tubules might be less responsive to aldosterone. Urine volume, creatinine clearance, and filtered Na\(^+\) load were virtually unaltered by aldosterone administration. As expected, aldosterone administration reduced Na\(^+\) and osmotic clearances, urine Na\(^+\)/K\(^+\) ratio, and total renal Na\(^+\) excretion and increased free water clearance during the control experimental condition. However, against expectations, renal responses to aldosterone infusion during HDT were similar to those measured during the control treatment. These results support the notion that renal distal tubular responsiveness to aldosterone was not altered by exposure to simulated microgravity.

Under our experimental conditions, aldosterone administration resulted in a similar elevation in circulating plasma aldosterone without affecting plasma renin activity, AVP, or ANP for both HDT and control experimental conditions. Plasma volume increased slightly but gradually during the 4-h period after aldosterone administration, probably as a result of the small maintenance saline infusion used to assure that the animals remained hydrated throughout the experiment. However, similarities or differences in renal responses to aldosterone infusion between HDT and control conditions could not be explained by this slight hypervolemic effect because the small elevation in plasma volume was similar between HDT and control. Therefore, it is unlikely that our interpretation of the effects of microgravity on interactions between aldosterone and renal tubular retention of Na\(^+\) were influenced by hemodynamic factors or responses of other hormones associated with regulation of body Na\(^+\) and fluid volume. Paradoxically, lower Na\(^+\) retention at baseline and throughout aldosterone administration during HDT was associated with a significant reduction, rather than an expected elevation, in circulating ANP. It is therefore conceivable that the renal tubules become more responsive to ANP and/or other natriuretic factors during adaptation to microgravity.

Our experimental conditions were not without potential limitations. Sedation by ketamine during the aldosterone infusion experiments was necessary to allow renal catheter placement, sequential collection of urine and blood samples, and measurements of hemodynamic responses. Ketamine has been shown to elevate hormones associated with fluid homeostasis (13), which could explain the elevated plasma levels of plasma renin activity and AVP. Ketamine can also act to elevate blood pressure and affect other hemodynamic responses (21), although heart rates and blood pressures in our animals during the experiments remained within normal ranges. Despite these potential compounding effects of ketamine on hormone and hemodynamic responses, anesthesia was identical for both HDT and control measurements, making it unlikely that differences between experimental conditions were affected by this drug. Although it was not measured in the present experiment, our previous measures of similar plasma cortisol in HDT and control conditions suggest that stress was controlled across the two experimental treatments. A major strength of this study was in its experimental design, in which each subject received both HDT and control conditions in random, counterbalanced order and all measurements were recorded in the 0° prone posture. This approach makes it unlikely that any differences observed under our experimental conditions could be explained by factors other than HDT.

Our results have implications for development of countermeasures against fluid and electrolyte loss during space missions and in the clinical environment. Attempts to restore circulating vascular volume with application of drinking or infusion of isotonic fluids have failed in humans confined to bed rest (5, 19) or exposed to spaceflight (22). Our results indicate that reduced renal Na\(^+\) retention probably contributes to this problem. Although pharmacological treatment with aldosterone or the fluoro derivative (e.g., Florinef) might be partly effective in increasing renal Na\(^+\) retention, our data suggest that total body Na\(^+\) excretion may remain elevated above normal baseline levels as a result of natriuretic mechanism(s) other than aldosterone. The identification of additional renal mechanism(s) that contribute to increased Na\(^+\) excretion during exposure to microgravity will be necessary for development of effective countermeasures for Na\(^+\) and water replacement.

The views expressed herein are the private views of the authors and not to be construed as representing those of the Department of Defense or Department of the Army. The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council. The Veterinary Sciences Re-
search Laboratory at Brooks AFB, TX, where the experiments were conducted, has been fully accredited by the American Association for Accreditation of Laboratory Animal Care since 1967.

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