Cardiovascular and Endocrine Responses to 90° Tilt During a 35-Day Saturation Dive to 46 and 37 ATA

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Claybaugh JR, Lin Y-C, Schafstall HG, Bennett PB. ***Cardiovascular and endocrine responses to 90° tilt during a 35-day saturation dive to 46 and 37 ATA*****. Aviat Space Environ Med 2007; 78:1042–9.**

Introduction: Hyperbaria-induced diuresis is accompanied by decreased basal and stimulated release of arginine vasopressin (AVP) and decreased blood volume possibly contributing to the reported orthostatic intolerance. Since hyperosmolality is not a consistent finding, the explanation of blood volume reduction at hyperbaria must involve an osmotic component to the diuresis. Investigations of a possible involvement of atrial natriuretic peptide (ANP) to the hyperbaric diuresis have revealed mixed results. ***Methods:*** Urinary excretion of electrolytes, AVP, and aldosterone were measured in four male subjects studied at 1 atmosphere absolute (ATA) and at 46 and 37 ATA (0.5 atmospheres pressure O_2: 5% N_2: remainder He) during a 35-d saturation dive. Also, the supine and 90° tilt-stimulated plasma levels of AVP, plasma renin activity (PRA), and aldosterone, and the suppressed responses of ANP and the cardiovascular responses to tilt were determined at these pressures. ***Results:*** Tilt-stimulated levels of PRA were increased two- to threefold and the AVP response was eliminated throughout hyperbaria, except in two episodes of tilt-induced syncope where AVP was elevated 10- to 20-fold. This pattern supports most previous reports. Contrary to some reports, both supine and tilt-suppressed levels of ANP were reduced by about 50% at all three tilt experiments conducted at hyperbaria compared to predive control values. ***Discussion:*** These results suggest an altered ANP response at pressures of 37 ATA or greater, which is consistent with an appropriate ANP response to blood volume reduction and further suggest that the hyperbaric diuresis is not dependent on increased ANP.

Keywords: hyperbaric diuresis, aldosterone, atrial natriuretic peptide, plasma renin activity, vasopressin, orthostatic hypotension.

EXPOSURE TO A hyperbaric environment results in reduced plasma volume (3,26) and evidence of orthostatic intolerance (1,12,14). The cause of the reduced plasma volume has often been linked to the well-documented diuresis that is apparent during exposure to atmospheric pressures of about 3 or more atmospheres absolute (ATA) (9). Although a reduced plasma level of arginine vasopressin (AVP) is a probable contributor to the dilute urine and diuresis (4), it may not be the whole explanation for the reduced plasma volume since plasma osmolality is not consistently increased (9). There must, therefore, be an associated loss of osmotic substances, resulting in an overall isosmotic volume reduction. Thus there has been a long-standing interest in the responses of salt regulating hormones to hyperbaria. Plasma renin activity (PRA) and aldosterone generally exhibit enhanced basal values during hyperbaric pressures of 18 to 31 ATA (2,9,15), and the head-up tilt (HUT)-stimulated release of PRA was greatly enhanced at 31 ATA (15). Therefore, the renin-angiotensin-aldosterone system has been thought to be responsive to the volume reduction rather than causal.

In support of a role for elevated levels of atrial natriuretic peptide (ANP) contributing to a natriuresis, the nocturnal urinary excretion of ANP has been reported to be increased during hyperbaric exposures to 16 and 21 ATA (16) and 26 and 31 ATA (28), and plasma levels of ANP have been reported to be increased sixfold with exposure to 3 ATA (21). However, ANP is also sensitive to central blood volume changes via atrial stretch (11). From this perspective, one would expect ANP to be decreased unless it was causal in the diuresis. In support of this view are the observations of lowered basal and exercise-stimulated release of ANP at 37 ATA (2). Other reports are inconsistent or demonstrate no response of ANP to hyperbaria during exposure to 61 and 46 ATA (17). Since orthostatic hypotension poses a potential danger in some individuals at hyperbaria, further information regarding its cause could be useful in appreciating the extent of the problem and developing strategies to safeguard against episodes. Among the proposed causes are the decreased plasma volume and the decreased AVP response to a change in posture to the upright position (1).

The present experiments were conducted nearly 16 yr ago at the GUSI (German Underwater Simulator) chambers at the Institut für Anlagentechnik in Geesthacht, Germany. The primary mission of that institute was to collaborate with private industry in the development

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This manuscript was received for review in November 2006. It was accepted for publication in August 2007.

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DOI: 10.3357/ASEM.2014.2007

and advancement of high-pressure technology and patentable products. We were allowed to use some of the professional divers who were also participating in those endeavors to do the experiments described in this manuscript. With the closure of highly productive hyperbaric research programs involving human experimentation in Germany and Japan, the likelihood that similar experiments would be conducted soon appeared remote. We, therefore, provide the data in this report in an attempt to extend and clarify the understanding of potential factors contributing to the hyperbaric diuresis and reduced plasma volume and, therefore, possibly to the orthostatic hypotension that develops at hyperbaria. The cardiovascular responses and the responses of the salt and water regulating hormonal systems to a 90° HUT were assessed in environments of 1, 46, and 37 ATA in order to determine if the orthostatic hypotension was exacerbated at the greater pressures of these experiments, and if there were more pronounced cardiovascular and endocrine differences in response to body fluid shifts.

METHODS

Subjects

Four male professional saturation divers volunteered to participate in the study. The studies described were approved by the Clinical Investigation Committee and the Human Use Committee at Tripler Army Medical Center, HI. The investigators adhered to all federal policies for the protection of human subjects. All subjects were informed and trained in the procedures and gave consent for participation. The studies were performed in the GUSI (German Underwater Simulator) chambers at the Institut für Anlagentechnik at the GKSS-Forschungszentrum in Geesthacht, Germany.

The subjects ranged in age from 34 to 39 yr, height from 173 to 191 cm, and weight from 77 to 94 kg. Their maximum heart rates ranged from 173 to 190 bpm and the maximum work load achieved on a bicycle ergometer was 300 to 400 W. Respiratory measurements were not available.

Dive Profile

Compression was begun on Dive Day (DD) 1 at 0800 reaching 46 ATA at 2000 on DD 2 (**Fig. 1**). The environmental gas was 0.5 ATA O_2, 5% N_2, and the balance He. The chamber remained at 46 ATA (gas density 10.3 kg · m^{-3}) until 2100 on DD 10, followed by a linear decompression to 37 ATA (gas density 8.4 kg · m^{-3}) reached at 1800 on DD 14. On DD 18 decompression recommenced reaching 1 ATA on DD 35. There were 24-h urine collections (day, 0700–1900; night, 1900–0700) done on DD −2 and −1 while at 1 ATA, DD 3 and 10 while at 46 ATA, DD 16 at 37 ATA, and DD 32 and 33 while near sea level (NSL, 48-h linear decompression from 8 to 3 ATA). Tilt table experiments were performed during the mornings of DD −1, 0, 3, 11, 15, 32, and 33 at pressures corresponding to the days of urine collections, except day 11, which was a day of decompression from about 45 to 43 ATA.

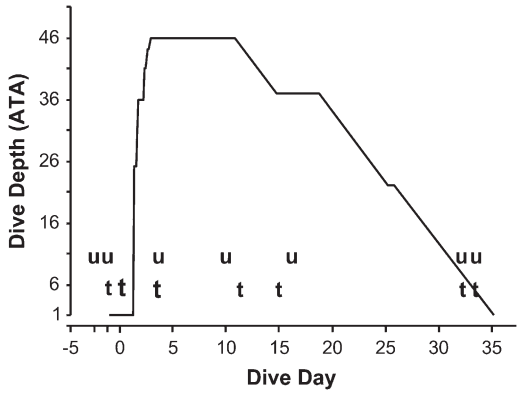

Fig. 1. Pressure profile and experimental schedule of the GUSI XVIII saturation dive to 46 ATA. Two urine collections were made from 0700 to 1900 and then from 1900 to 0700 on the Dive Days designated by a "u." Tilt table studies were on the days designated by a "t."

Experiments

The tilt table experiments were conducted in the same manner as previously conducted at 31 ATA (15) in order to provide some comparability of the responses. The subjects inserted intravenous catheters into one another's arm veins while in the seated position prior to the experiments. The catheters were filled with heparinized saline and affixed with a three-way stopcock to aid in obtaining blood samples and avoid the pain of venipuncture during the experiment. On the opposite arm, a blood pressure cuff was positioned over the radial artery for measurement of pulse rate and arterial blood pressure. One at a time, the subjects laid quietly on the tilt table in the supine position with their bodies strapped to the platform and their feet positioned on a footrest at a right angle from the table. After 10 min in this position, systolic and diastolic arterial blood pressures and pulse rate were recorded and 22 ml of blood was withdrawn and distributed to a heparinized vacutainer (12 ml) and a sodium EDTA-containing vacutainer (10 ml) and placed on ice. The table was then manually tilted to 90° within 5 s, placing the subject in the upright position. The subject remained motionless while resting his feet on the footrest and strapped to the table. The strapping was provided to aid the subject in remaining motionless and as a precaution in the event of a fainting episode, but also suspended the subjects to minimize leg muscle contraction and venous return. Subjects were closely observed throughout. After 15 min in the upright position, a second set of measurements and blood samples were obtained. When subject experienced syncope, that is, lost consciousness, the subject was immediately returned to the supine position, and the blood sample taken in that position. All of the experiments were conducted in the morning.

Changes in plasma volume in response to tilt were determined using the changes in hematocrit according to the calculation determined by van Beaumont (31) as follows:

$$\%\Delta PV = (100/100 - Hct_1)\cdot(100\cdot(Hct_1 - Hct_2))/Hct_2$$

Where %ΔPV is the % change in plasma volume, Hct_1 is the hematocrit in the supine position, and Hct_2 is the hematocrit in the upright passive standing position. Orthostatic tolerance was assessed grossly by the incidence of syncope.

At the conclusion of all experiments, requiring approximately 3 h, all blood samples kept on ice were locked out of the chamber and slowly decompressed to 1 ATA over a period of approximately 45 min. The slow decompression was done to eliminate foaming and hemolysis (26). The blood in the heparinized sample tubes was gently mixed to redistribute the erythrocytes, and a sample was removed for determination of hemoglobin and hematocrit at a commercial clinical laboratory by Coulter counter methodology. The remainder of the blood was centrifuged at 1500 g for 20 min, and the plasma separated. The heparinized plasma was used for AVP, aldosterone, electrolyte, and osmolality determinations, and the EDTA plasma was used for PRA and ANP assays.

Each day of collection, 12-h urine collections were locked out soon after 0700 and 1900. However, intakes of calories, electrolytes, and fluids were not obtained. Furthermore, the total urine volumes were not recorded on the first 2 d of collection and, therefore, urine flow was not measured throughout, and all urinary values are expressed as a function of creatinine excretion.

Plasma and urine samples were frozen at –20°C until the study was completed, and transported on dry ice to Tripler Army Medical Center where they were kept at –20°C until analyses were completed. Plasma and urinary sodium and potassium concentrations were measured by flame photometry (Instrument Laboratory, model 643, Lexington, MA), osmolality by freezing point depression (Advanced, Model 3D2, Needham, MA), and creatinine by the Jaffe reaction (Beckman Astra-4 Autoanalyzer, Brea, CA). All plasma hormonal assays were done by radioimmunoassay as previously described (2,5).

Statistics

The number of subjects was not established through a statistical power analysis. Rather, the intent was to detect changes, if they occurred, between different stages of the study among the four subjects, with predive and NSL periods control periods. Power to detect such differences is improved by the addition of more measurement times. The power of detecting a change of 30% in any mean value over the three time periods at hyperbaria, assuming a 30% SD about each of the seven means, with four subjects, was calculated to be 95% using a one-way analysis of variance with repeated measures over the seven time periods. In order to determine differences between individual means, a post hoc Student's *t* least significant difference test was used. In addition, orthogonal comparisons were used to compare the multiple values in the predive period with the multiple values in other periods. The AVP values and cardiovascular responses associated with syncope were not included in the statistical analysis, nor in the means of the tables or figures, and the degrees of freedom were decreased accordingly. The data were analyzed using JMP statistical software (version 4, SAS Institute, Cary, NC).

RESULTS

Daytime and nighttime excretion of AVP was equivalent throughout the dive, but the daytime excretion of the hormone was decreased at hyperbaria compared to the predive period, which continued through the NSL collection period (**Table I**). The nighttime values showed a similar pattern, but the significant decrease compared to the predive values did not occur until the NSL period. As expected, the decreased daytime AVP excretion was accompanied by decreased urine osmolality. The nighttime urine osmolality was lower than the daytime at predive, which we attribute to an unusual subject, who apparently drank excess fluids on both nights during the predive period, resulting in a reduction in urine creatinine values between daytime and nighttime collections of 80%. This would suggest an unusual fivefold increase in urine flow at night. Without fluid intake values, this irregularity is without definitive explanation. Since the mean osmolality values were already low during the predive period, a further decrease at hyperbaria would

TABLE I. MEAN (± SEM) URINARY EXCRETION OF ARGININE VASOPRESSIN (U_{AVP}), ALDOSTERONE (U_{ALDO}), SODIUM (U_{NA}), POTASSIUM (U_K), AND URINE OSMOLALITY (U_{OSM}).

Measurement	Collection Period	Pre-Dive		Dive			NSL		Pre vs. 3–16†	Pre vs. 32–33	Pre vs. 3–33
		DD −2	DD −1	DD 3	DD 10	DD 16	DD 32	DD 33			
U_{AVP}	Day	30 ± 14	21 ± 2	12 ± 1	9 ± 2+	12 ± 5+	7 ± 1+	10 ± 1+	0.0292	0.0132	0.01
(mU · mg^{-1} Cr)	Night	17 ± 3	18 ± 3	22 ± 12	8 ± 1	10 ± 2	6 ± 1	7 ± 1		0.0367	
U_{ALDO}	Day	11 ± 3	11 ± 4	8 ± 2	5 ± 1+	13 ± 4	11 ± 2	9 ± 1			
(ng · mg^{-1} Cr)	Night	4 ± 1*	4 ± 1*	4 ± 1	3 ± 1	5 ± 2*	5 ± 1*	4 ± 1			
U_{OSM}	Day	829 ± 82	853 ± 107	613 ± 79+	526 ± 48+	654 ± 154	548 ± 148+	472 ± 94+	0.0009	0.0002	0.0001
(mOsm · kg^{-1})	Night	542 ± 94*	642 ± 144	595 ± 139	666 ± 114	591 ± 126	526 ± 70	530 ± 33			
U_{Na}	Day	88 ± 32	95 ± 21	75 ± 32	80 ± 17	31 ± 5+	49 ± 6+	92 ± 12			
(mEq · mg^{-1} Cr)	Night	107 ± 32	103 ± 12	88 ± 19	96 ± 15	76 ± 12	63 ± 11	66 ± 7		.0311	
U_K	Day	68 ± 3	77 ± 6	61 ± 6	36 ± 4+	29 ± 4+	44 ± 6+	55 ± 7	0.0001	0.0001	0.0001
(mEq · mg^{-1} Cr)	Night	37 ± 7*	27 ± 6*	46 ± 2	34 ± 5	47 ± 4*	26 ± 4*	25 ± 3*	0.0144		

NSL = near sea level.
† = Orthogonal comparisons of predive days –2 and –1 with the block of dive days indicated; values in these columns are probability (*P*) values relative to the indicated period of the corresponding row; only *P*-values less than 0.1 are shown.
* = $P < 0.05$ compared to corresponding Day value. + = $P < 0.05$ compared to either Dive Day (DD) –2 or –1 of the corresponding row.

be less likely and probably accounts for the atypical response.

The urinary excretion of aldosterone revealed a strong circadian pattern with daytime greater than nighttime, which disappeared during the two days of collection at 46 ATA, but returned after decompression to 37 ATA. This loss in circadian rhythm was due to a significant decrease in the daytime excretion of aldosterone at 46 ATA compared to the predive ($P < 0.05$, orthogonal comparison not shown).

The excretion of sodium exhibited no circadian pattern and tended to decrease throughout the study, being significantly reduced during the NSL period in the nighttime collection (Table I). Urinary excretion of potassium, on the other hand, revealed a clear circadian pattern during the predive period, reflecting urinary aldosterone excretion, with the daytime excretion greater than the nighttime. Subsequently both urinary excretory patterns disappeared during the early exposure to 46 ATA, with a return of aldosterone to normal values at 37 ATA, and the potassium pattern reversed at 37 ATA, but was restored to normal during the NSL urine collections with daytime excretion of potassium again greater than nighttime. Plasma osmolality was increased in both the supine and upright tilt positions during all three experiments at hyperbaria, compared to values at predive (**Table II**).

Similarly, plasma sodium concentration was increased during the experiments at 37 ATA. The supine value of plasma potassium was increased on the second tilt experiment conducted at 46 ATA, which resulted in a difference between the supine and upright values. This was the highest value seen in three of the four subjects, and with no obvious physiological explanation, we can only ascribe the cause to an unknown technical problem such as a slightly extended storage on ice before centrifugation or slight hemolysis undetectable from hematocrit values.

We observed two episodes of syncope. One occurred in response to HUT in the second set of tilt experiments conducted at 46 ATA, and the other occurred in the same subject in the experiments conducted at 37 ATA. The syncope was associated with plasma AVP levels of 7.4 and 10.6 $\mu U \cdot ml^{-1}$, which were many fold higher than the mean values of the remaining subjects (**Fig. 2**). These values were not included in the statistical analysis of the AVP response.

HUT during predive was associated with an increase in hematocrit and decrease in plasma volume by about 11% (Table II). The percent decrease in plasma volume was 19% in response to HUT during the first tilt experiments at 46 ATA, when the supine hematocrit was highest and, therefore, the basal plasma volume was likely the lowest. The change in plasma volume in response to HUT tended to be greater during all periods at hyperbaria ($P = 0.0227$, Table II), but the response was normalized in the NSL experiments.

The cardiovascular responses to HUT revealed an increased heart rate, maintained systolic blood pressure, and increased diastolic pressure in all periods of the study (**Table III**). Virtually all significant changes in the tilt values at hyperbaria were in association with parallel changes in the supine and HUT values and, therefore, the magnitude of changes was similar. Thus the calculated cardiovascular index of deconditioning did not change with exposure to hyperbaria (data not shown).

Vasopressin was stimulated by HUT during both predive tilt experiments, but this response was eliminated during hyperbaria (Fig. 2). In contrast, the modest PRA response to HUT did not reach statistical significance, but was greatly exaggerated as exposure to hyperbaria continued, with HUT-stimulated values greater than predive stimulated values at 37 ATA with 15 d exposure to higher pressures. This exaggerated response of PRA to HUT continued through the first tilt experiments conducted at NSL, but returned to predive response levels in the tilt experiments conducted the next day. The response of plasma aldosterone to HUT was slight and not statistically significant in the predive experiments and in those conducted at 46 and 37 ATA. Additionally, the values of aldosterone following HUT on the first day at 46 ATA were significantly reduced compared to the predive responses, and were not significantly enhanced with extended exposure to hyperbaria,

TABLE II. MEAN (± SEM) VALUES OF PLASMA OSMOLALITY (P_{OSM}), SODIUM (P_{NA}), AND POTASSIUM CONCENTRATION (P_K), AND HEMATOCRIT (HCT) IN SUPINE (S) AND 90° HEAD-UP TILT (HUT) POSITIONS, AND CHANGE IN PLASMA VOLUME (ΔPV) IN RESPONSE TO HUT AT PREDIVE, DIVE, AND NEAR SEA LEVEL (NSL).

		Pre-Dive		Dive			NSL				
Measurement	Position	DD −1	DD 0	DD 3	DD 11	DD 15	DD 32	DD 33	Pre vs. 3–15	Pre vs. 32–33	Pre vs. 3–33
P_{OSM}	S	300 ± 2	298 ± 2	306 ± 2+	302 ± 3	312 ± 2+	300 ± 2	300 ± 3	0.0009		0.0109
(mOsm · kg^{-1})	HUT	303 ± 1	298 ± 1	311 ± 2+	306 ± 2+	312 ± 1+	301 ± 2	303 ± 3	0.0001		0.0001
P_{Na}	S	147 ± 1	145 ± 1	147 ± 1	146 ± 1	151 ± 1+	146 ± 1	146 ± 1	0.035		
(mEq · L^{-1})	HUT	148 ± 1	146 ± 1	148 ± 1	147 ± 1	152 ± 1+	146 ± 1	146 ± 1	0.0034		0.0901
P_K	S	3.9 ± 0.1	4.0 ± 0.1	3.6 ± 0.2	4.6 ± 0.4+	3.7 ± 0.4	3.8 ± 0.1	3.8 ± 0.1			
(mEq · L^{-1})	HUT	4.2 ± 0.2	4.1 ± 0.1	4.0 ± 0.3	3.9 ± 0.4*	3.6 ± 0.1	4.0 ± 0.1	4.0 ± 1			
Hct	S	45 ± 1	45 ± 1	48 ± 1+	45 ± 1	46 ± 1	43 ± 1	42 ± 1	0.0104	0.0207	
(% RBC)†	HUT	47 ± 1*	48 ± 1*	53 ± 2*	48 ± 1*	51 ± 1*+	45 ± 1*	46 ± 2*	0.001		0.0712
ΔPV (%)		−11 ± 2	−11 ± 1	−19 ± 1+	−14 ± 3	−19 ± 2	−10 ± 2	−14 ± 4	0.227		0.813

† = % blood volume composed of erythrocytes determined by Coulter counter. Orthogonal comparisons are as described for Table I.
* = $P < 0.05$ compared to corresponding Supine value. + = $P < 0.05$ compared to either Dive Day (DD) −2 or −1 of corresponding row.

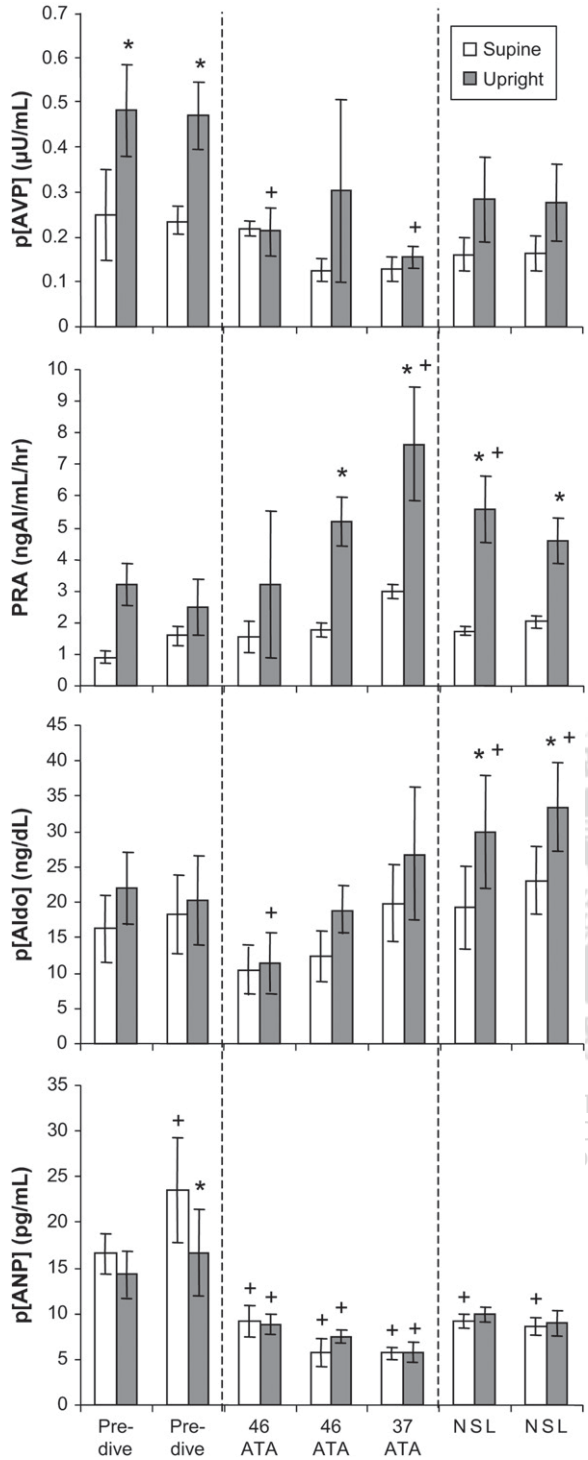

Fig. 2. The vertical histogram bars represent mean values, and the error bars are ± 1 SE of the mean. p[AVP] = plasma arginine vasopressin concentration ($\mu U \cdot ml^{-1}$; 1 μU = 2.5 pg = 2.3 fMole); PRA = plasma renin activity (ng angiotensin I generated per ml plasma per hour at 37°C at pH 5.6); p[aldo] = plasma aldosterone concentration ($ng \cdot dl^{-1}$); p [ANP] = plasma atrial natriuretic peptide concentration ($pg \cdot ml^{-1}$). * = $P < 0.05$ compared to supine value on the same day, + = $P < 0.05$ compared to either or both predive values.

as was seen with the PRA response. This apparent uncoupling between PRA and aldosterone responses is further evidenced during the NSL exposure period where the PRA response to HUT was becoming normalized, but the plasma aldosterone response was the most pronounced.

The inhibition of ANP in response to HUT was statistically significant only during the second set of predive tilt experiments. Both supine and upright values of plasma ANP were significantly reduced at all three experiments conducted at 46 and 37 ATA. During the NSL period, the HUT-inhibited values were no longer significantly lower than the predive values.

DISCUSSION

Upright tilt at hyperbaria in the present studies was associated with orthostatic intolerance, as evidenced by two episodes of syncope in the same individual on DD 11 and 15. In an attempt to determine if there were generalized cardiovascular changes that could predict orthostatic intolerance, or cardiovascular deconditioning that takes place at hyperbaria, we calculated the cardiovascular index of deconditioning in the analysis of the present data (data not shown), but a significant increase in this measurement could not be documented as previously demonstrated at 31 ATA (1,12). Our results, therefore, did not reveal a generalized pattern, but confirm that hyperbaria increases the susceptibility of some individuals. The subject that experienced syncope in the present studies was of similar fitness, as could be judged by a maximum power output of 300 W compared to a mean of 338 W for the group, and his height and weight were within range of the others. He was 3 to 4 yr older than the others, at 39 yr of age, and his resting hematocrit values in the supine position were always the highest of the four divers, with only one exception, suggesting a relatively reduced plasma volume and possibly a lesser state of training. Similar to a previous study (1), the incidences of syncope only occurred at hyperbaria and, therefore, would not appear to be associated with extended periods of reduced activity or confinement. The lack of evidence for worsening cardiovascular responses or plasma volume changes to the HUT maneuver argues against greater orthostatic intolerance at the greater pressures studied in the present work.

The present studies were also conducted to further clarify the hormonal responses that could better explain the hyperbaric diuresis. A hyperbaric diuresis could not be documented in the present study; however, daytime urine osmolality and AVP excretion and plasma concentrations were decreased during hyperbaria as shown in previous dry saturation dives to 31 ATA (3,4,6,22,26). The decrease in urine osmolality, most likely due to the reduced AVP, appears to be decreased in a pressure-dependent manner when data from several published studies were analyzed (8). The primary mechanism for the decreased AVP was initially thought to be a direct consequence of reduced insensible water loss, which, when coupled with a constant water intake, lead to a reduced plasma osmolality and decreased AVP, causing a free water diuresis (10). However, more recent studies have demonstrated that even with an acute 60-min exposure to as little as 3 ATA in air, with gas density at

TABLE III. MEAN (± SEM) VALUES OF HEART RATE (HR), ARTERIAL SYSTOLIC BLOOD PRESSURE (SBP), ARTERIAL DIASTOLIC BLOOD PRESSURE (DBP), AND MEAN ARTERIAL BLOOD PRESSURE (MBP), IN RESPONSE TO SUPINE (S) AND 90° HEAD-UP TILT (HUT) AT PREDIVE, HYPERBARIA, AND NEAR SEA LEVEL (NSL).

		Pre-Dive		Dive			NSL		Pre vs.	Pre vs.	Pre vs.
Measurement	Position	DD −1	DD 0	DD 3	DD 11	DD 15	DD 32	DD 33	3–15	32–33	3–33
HR	S	61 ± 2	64 ± 2	54 ± 3	66 ± 2	62 ± 4	71 ± 4	67 ± 3			
(bpm)	HUT	74 ± 5*	74 ± 3	71 ± 11*	73 ± 4	69 ± 5	91 ± 6*$^{+}$	83 ± 4*		0.0481	
SBP	S	120 ± 4	121 ± 5	115 ± 2	144 ± 4^{+}	126 ± 4	120 ± 4	118 ± 3	0.0045		0.0641
(mmHg)	HUT	118 ± 4	119 ± 4	118 ± 4	143 ± 3^{+}	125 ± 1	117 ± 4	113 ± 3	0.0003		0.0326
DBP	S	76 ± 1	74 ± 1	73 ± 4	90 ± 7^{+}	85 ± 3^{+}	76 ± 1	74 ± 2	0.0381		
(mmHg)	HUT	86 ± 3*	84 ± 3*	90 ± 6*	94 ± 3	91 ± 3	85 ± 2	83 ± 4			
MBP	S	90 ± 2	89 ± 2	87 ± 3	108 ± 5^{+}	98 ± 3^{+}	91 ± 2	89 ± 2	0.0104		0.0726
(mmHg)	HUT	97 ± 4	96 ± 2	99 ± 5*	111 ± 2^{+}	102 ± 2	96 ± 3	93 ± 3	0.0146		

The table arrangement and symbols are as described for Table II.

approximately 3.3 kg · m^{-3}, a decrease in plasma AVP will result, thus ruling out a requirement for extended periods of decreased insensible water loss and accumulation of free water (29).

Gas density may also play a role in the diuresis by causing negative pressure breathing, consequent increase in thoracic blood volume, and increased cardiac volume receptor stretch. The threshold of gas density that is able to cause a diuresis is about 3.8 kg · m^{-3}, above which a diuresis will occur (18,20). For instance, no diuresis occurred at 4 ATA in the He-O_2 environment at a gas density of 1.82 kg · m^{-3} (27), but did occur at 3 ATA in a N_2-O_2 environment at 3.79 kg · m^{-3} (23), and a diuresis did not occur at 2.5 ATA in a N_2-O_2 environment at 3.16 kg · m^{-3} (18). In the present dive, the gas density at 37 and 46 ATA was 8.4 and 10.3 kg · m^{-3}, respectively, well above the threshold. However, negative pressure breathing induced by placing a mechanical resistance on the inspiration phase of respiration to a similar degree of negative pressure, as would be caused by these gas densities, also leads to reduced plasma renin activity and increased ANP (7). Therefore, the diuresis that is dependent upon increased gas density at slightly elevated environmental pressures may involve a natriuretic component caused by these hormonal responses, unlike the increased renin-angiotensin-aldosterone activity reported at pressures greater than 18.6 ATA (9).

As a final consideration for the mechanism of the reduced AVP release at hyperbaria, the direct effects of pressure or the partial pressure of one of the gases cannot be ruled out. Experiments by Osaka et al. (19) demonstrated that exposure of conscious rats to 7 ATA in air reversibly reduced the firing rate of approximately two-thirds of the hypothalamic neurons, while most neurons in the prefrontal cortex and central medial thalamus remained unaffected. Such findings suggest more direct effects of hyperbaria, or perhaps increased partial pressures of oxygen or nitrogen specifically, on vasopressinergic responses. Thus, factors other than plasma osmolality and the effects of increased gas density may play a role in the hyperbaric suppression of AVP release and the attendant diuresis.

The AVP and PRA responses to HUT at 46 and 37 ATA in the present study were remarkably similar to responses previously observed at 31 ATA (15). That is, the AVP response was abolished during hyperbaria, and the PRA response, although similar to sea level responses in the early exposure period, became significantly enhanced as the hyperbaria continued. Both the AVP and PRA responses returned toward predive values during the NSL period, which is also similar to the previous studies. The decreased basal AVP has been discussed above, and most likely explains the diminished response to HUT at hyperbaria. The enhanced PRA response seen in the present dive and others (4,15) is difficult to fully explain. On the one hand, plasma volume is reduced and may contribute to an exaggerated response, but this decrease in plasma volume has been shown to be transient with recovery after 7 d at 31 ATA (26). However, the PRA responses to HUT were greatest at that time (15), similar to those responses in the present dive. The enhanced response is not likely due to enhanced sympathetic stimulation of renin release because direct measurements of sympathetic nerve activity in response to lower body negative pressure were reduced at 3 ATA, as well as basal sympathetic activity and plasma norepinephrine levels (33). Furthermore, at 31 ATA, HUT was associated with a reduced increase in total peripheral resistance compared to the responses at 1 ATA, also supporting a reduced sympathetic response to tilt at hyperbaria. Since AVP is an effective inhibitor of renin release (13,24), removal of the vasopressin inhibition probably contributes to the enhanced PRA response we observed in the present study. Also contributing to the increased PRA may be the reduced plasma levels of ANP we observed throughout hyperbaria. Infusions of ANP, for instance, have been shown to reduce PRA despite decreases in mean arterial blood pressure by 36 mmHg in spontaneously hypertensive monkeys (30); therefore, greatly reduced ANP levels would favor an enhancement of renin release.

Unlike the AVP and PRA responses, the aldosterone responses in the present study were notably different than the previous studies. In the previous experiments performed at pressures between 18 and 31 ATA, the urinary excretion of aldosterone as well as plasma levels of the hormone increased during hyperbaric exposure, corresponding to increased PRA (9). The increased urinary

aldosterone excretion occurred during both daytime and nighttime (4). The present results are in contrast with those previous studies since daytime urinary excretion of aldosterone decreased during the 46 ATA exposure, and recovered to baseline values when the chamber pressure was reduced to 37 ATA, while nighttime values did not change over time. The plasma values of aldosterone tended to be lower at 46 ATA and thus reflected the daytime urinary excretion of the hormone, providing confirmation of the daytime response. The decrease in daytime urinary excretion of aldosterone caused the typical circadian rhythm to be lost at 46 ATA, and in general, periods of decreased aldosterone were accompanied by decreased urinary potassium excretion, which is also contrary to most studies, which report an increased urinary potassium excretion at hyperbaria (9).

Most remarkable is the clear uncoupling between plasma aldosterone and PRA that occurred during the NSL tilt experiments in the present experiments. The modest tilt-induced stimulation of aldosterone at 1 ATA in the predive control period and during the entire dive period was not increased significantly in any individual experiment, which is nearly identical to responses in previous experiments where plasma aldosterone was measured in response to tilt at 31 ATA. However, during the NSL experiments, the plasma levels of aldosterone were significantly elevated in both tilt experiments despite a reduced PRA response. Altered electrolyte responses to the tilt were not detected over the course of the study and are, therefore, not likely contributors to the heightened aldosterone response during the NSL experiments.

The present studies confirm our earlier observations of decreased basal plasma ANP concentration at hyperbaric exposures to 37 and 46 ATA, and further demonstrate that not only the exercise-stimulated levels are decreased, as determined previously (2), but also the HUT-suppressed levels are decreased. The plasma levels were consistently suppressed for all six samples taken at hyperbaria in the present study. Two studies have reported that exposure to hyperbaria ranging from 16 to 31 ATA increased the urinary excretion of ANP and, importantly, the increase occurred only at night (16,28), concurrent with the hyperbaria-induced diuresis which is most prevalent at night (9). In both of those studies, the daytime ANP excretion was decreased at hyperbaria, albeit not to a statistically significant degree. We are unaware of any reports where plasma ANP has been measured at night, and none of the reports of daytime plasma ANP measurements have shown a consistent pattern of increased or decreased ANP levels at atmospheric pressures of 16 ATA or greater (16,17,22). Given the fact that the kidney is a major metabolism site of ANP (32) and that plasma levels and urinary levels can respond in opposite directions (25), assessment of urinary levels of the hormone to estimate integrated plasma levels is subject to pitfalls and must be interpreted cautiously. Nevertheless, there may be a diurnal pattern of ANP at hyperbaria, such that nighttime levels are exaggerated and daytime levels are suppressed, although this will require confirmation with analysis of plasma levels. At lesser hyperbaric pressures of 2 and 3 ATA in air, and with brief 5-min exposures, a pressure-dependent sixfold increase in ANP has also been reported in human subjects, but the acute nature of the study precludes meaningful comparisons to the prolonged exposures of other studies, and is not in agreement with one other study of prolonged exposure at 2.5 ATA, where no change in plasma ANP was observed.

We currently have no explanation for the observed decreased ANP levels in this study and the previous study by our group (2). The Trimix gas mixture used in the present studies was similar to that used in the previous dives to 61 and 46 atm reported by Moon et al. (17), and not greatly different from the studies done at 16 and 21 ATA (16). Differences in posture would not seem to be important because we have observed decreased ANP at hyperbaria in the supine and upright passive standing position in the present studies, and in the seated resting condition and exercise conditions previously (2); the decreased ANP persisted throughout the course of hyperbaria in both studies. A decrease in ANP would be expected during a volume depleted state, which has been documented extensively in hyperbaric exposures to pressures used in these studies and others (3,26). Lastly, the enhanced PRA response late in the hyperbaric exposure is consistent with decreased ANP levels and decreased vasopressin levels. Taken together, the ANP results are internally consistent and consistent with concurrent body fluid and hormonal status, and consistent with an earlier study at a similar atmospheric pressure, but inexplicably not in agreement with other previous reports.

Conclusions

The present studies reveal a similar pattern of orthostatic intolerance as previously reported during HUT at hyperbaric exposures, and although these tilt table experiments were conducted at the greatest pressures to date, there was no evidence of a greater degree of orthostatic tolerance. Similarly, the hormonal responses of AVP and PRA to the 90° HUT at 46 and 37 ATA in the present studies were remarkably similar to the results of identical studies conducted at 31 ATA (15), and the ANP response to hyperbaria was reduced as reported previously by our laboratory (2). The primary difference noted in these studies was the response of plasma concentrations of aldosterone. Plasma aldosterone revealed an atypical tendency for decreased levels at hyperbaria, and then an uncoupling with PRA and exaggerated tilt-induced increase during the late decompression phase at NSL. It is hoped that further investigation into these salt-regulating hormones with superimposed stimuli such as exercise, or head up or head down tilt, or perhaps immersion, will be studied to characterize the response of these hormone systems at hyperbaria.

Lastly, as with most deep saturation dive studies, the number of subjects in the present study was limited. The statistics applied can only determine if a response over time among those limited subjects, in this case four, is

likely or not likely to be accounted for by chance; the statistical power is generated by taking several repeated samples in the same subjects in various phases of the dive. The risk of generalizing the data to the overall population becomes greater as the studied population deviates from the overall population, and with the presence of unaccounted for experimental factors that affect measured variables.

ACKNOWLEDGMENTS

The authors are indebted to the GKSS institute that bore the major expenses of the project and to the support staff who expertly manned the chambers and tended to the subjects' needs. We are very grateful for the diligence with which the subjects conducted all of the tasks of urine collection, intravenous catheterization and blood sampling, and tending to one another during the experiments. We also thank Mrs. Aileen Sato and Sgt. Elsa E. Piña for their expert technical assistance in the radioimmunoassay of the hormones.

Support was received from the U.S. Army Health Services command, and NOAA (grant NA88AA-D-URO48).

The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the U.S. Government.

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