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Hyperbaric Diuresis is Associated with Decreased Antidiuretic Hormone and Increased Atrial Natriuretic Polypeptide in Humans

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Abstract When men are exposed to a hyperbaric environment, urine flow increases. In order to elucidate the mechanism of this hyperbaric diuresis, a dry saturation dive experiment was carried out. Five male subjects were exposed to a 16–21 ATA (atmospheric pressure absolute) helium-oxygen (He-O_2) environment for 4 days. Five blood samples were obtained in the early morning (0600–0630 h): once at predive 1 ATA air, 3 times at 16–21 ATA He-O_2, and once at postdive 1 ATA air. Eight-hour timed urine samples, 0600–1400 h, 1400–2200 h, and 2200–0600 h (night urine), were collected throughout the experimental period. Urine flow markedly increased by the exposure to hyperbaria in the presence of constant creatinine clearance. The increase was mostly attributable to the urine flow during 2200–0600 h. The secretion of antidiuretic hormone (ADH) was suppressed at daytime and night during the exposure. On the other hand, the secretion of atrial natriuretic polypeptide (ANP) increased solely at night during hyperbaria and correlated with the increases of both the nocturnal urine flow and the nocturnal urinary excretion of sodium. These results suggest that both suppressed ADH secretion and stimulated ANP secretion cause hyperbaric diuresis.

Key words: hyperbaria, diuresis, night urine, ADH, ANP.

A hyperbaric environment causes various metabolic alterations in men. Among them, increased urine flow called hyperbaric diuresis, which occurs at above 4 ATA (TAMURA *et al.*, 1983; NIU *et al.*, 1990), is one of the major problems to divers because hemoconcentration is apt to cause decompression sickness (WEIHL, 1978). This hyperbaric diuresis has been observed in many investigations employing saturation dive (BENNETT and GRAY, 1971; ALEXANDER *et al.*, 1973; MATSUDA *et*

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al., 1975; HONG et al., 1977; RAYMOND et al., 1980; SHIRAKI et al., 1987). Though the mechanism of the diuresis is not fully understood, several factors have been proposed as causes of the diuresis. They include cold stress (BENNETT and GRAY, 1971), osmotic gas gradients (SCHAFFER et al. 1970), augmented negative pressure breathing (WRIGHT et al., 1973; HONG, 1975), and suppression of insensible water loss (HONG, 1975; HONG et al., 1977). HONG et al. (1977) presumed that suppression of insensible water loss was the primary cause for the diuresis. In either case, these conditions lead to an increase of intra-thoracic blood volume, resulting in suppression of ADH secretion and subsequent water diuresis. The important role of ADH in hyperbaric diuresis has been pointed out by various investigators (LEACH et al., 1973; HONG et al., 1977; CLAYBAUGH et al., 1984, 1987). However, it was also indicated that hyperbaric diuresis often accompanied natriuresis (HONG, 1975), and an osmotic factor was important for the diuresis (MATSUDA et al., 1975, 1978; HONG et al., 1977; NEUMAN et al., 1979; SHIRAKI et al., 1985). CLAYBAUGH et al. (1984) reported that ADH secretion was reduced during decompression while urine flow decreased. These observations suggested that some other factor(s) than ADH, such as "third factor" or "natriuretic factor," might be involved in the diuresis at high pressure (HONG, 1975; NAKAYAMA et al., 1981; HONG et al., 1983; SHIRAKI et al., 1985, 1987; CLAYBAUGH et al., 1987).

As the pressure employed has become higher and urinalysis more extensive in a dive experiment to clarify the characteristics of hyperbaric diuresis, a new aspect of the diuresis has been noticed. NAKAYAMA et al. (1981) observed for the first time that increased urine flow during night accounted for the major part of the diuresis in a 31 ATA experiment named "Seadragon IV" conducted at the Japan Marine Science and Technology Center. This nocturnal diuresis was also observed in the following experiments at 31 ATA named "Seadragon V and VI" (SHIRAKI et al., 1984, 1987) or at 4 ATA named "Sea Mecca I" (TAMURA et al., 1983). Besides, it was indicated that hyperbaric diuresis consisted of two components, namely water diuresis and osmotic diuresis and that the latter was characteristic in the night urine (SHIRAKI et al., 1987). Thus, it has become indispensable to inquire into the "third factor" or "natriuretic factor" in order to understand the whole figure of hyperbaric diuresis.

ANP is a hormone which is secreted from cardiocytes of the atria when atrial pressure is elevated (DE BOLD et al., 1981). Since this hormone acts on renal tubules to cause diuresis and natriuresis (CANTIN and GENEST, 1985), the change of its secretion may give us a clue for elucidation of the mechanism of hyperbaric diuresis. There are a few reports on dive experiments including the changes in plasma ANP levels in the morning (MOON et al., 1987; NIU et al., 1990; SAGAWA et al., 1990). However, they could not show a clear concern of ANP with hyperbaric diuresis. In order to know the mechanism of the diuresis, it would be necessary to draw clues from night samples of blood or urine since nocturnal diuresis composes the major part of hyperbaric diuresis. In the current study, we determined ANP levels not only in blood in the morning but also in urine including nighttime.

SUBJECTS AND METHODS

Experimental protocol. The experiment was carried out in a hyperbaric chamber at Undersea Medical Center of Japan Maritime Self-Defence Force at Yokosuka, Japan. The experimental protocol is shown in Fig. 1.

Six healthy male adults, 22–37 years old, were the subjects of this dry saturation dive experiment. They were informed of the experimental procedure and risks in advance and took part in the experiment with consent. Before compression, all the subjects entered the hyperbaric chamber for the predive 1 ATA control period (pre). Compression started at 0905 h on the dive Day 1, using air until 2 ATA and He-O_2 gas thereafter. After a stop at 16 ATA from 2025 h on the dive Day 1 to 0900 h on the dive Day 3, compression was recommenced and was completed to 21 ATA at 1040 h on the dive Day 3. Decompression started at 1600 h on the dive Day 4 and was completed at 1005 h on the dive Day 11. The subjects stayed in the chamber for 3 more days after decompression and the last day was designated as the postdive 1 ATA control period (post).

The ambient gas was maintained at 0.42–0.43 ATA O_2, 0.40–1.60 ATA N_2, and remaining helium when compressed above 2 ATA. CO_2 pressure was kept at approximately 0.005 ATA by circulating the chamber gas through a CO_2-absorbing unit. The ambient temperature was controlled at 27°C at 1 ATA and 30°C at 16–21 ATA. The relative humidity was 60–70%.

Blood samples were obtained from five subjects in the early morning (0600–0630 h), once at predive and postdive 1 ATA control periods, two times at

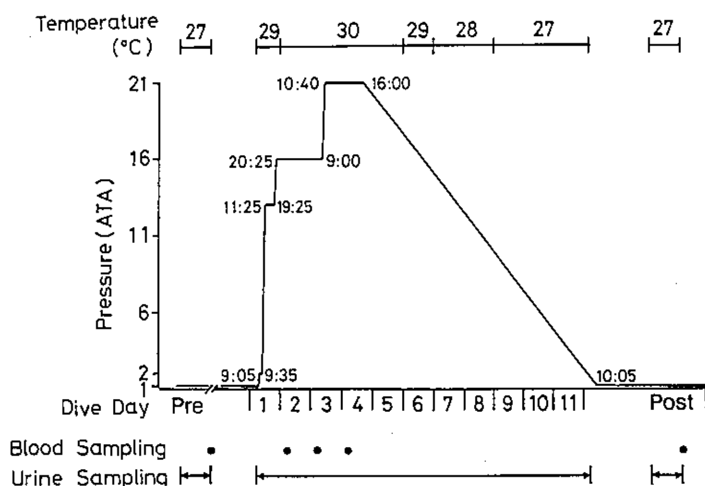

Fig. 1. Dive profile. The change of ambient pressure is shown. The temperature in the chamber is represented at the top of the figure. The days when venous blood samples (●) or urine samples (↔) were obtained are shown at the bottom of the figure.

16 ATA, and once at 21 ATA. In order to examine the difference in character of the urine between daytime and night, 8-h timed urine samples, 0600–1400 h (fraction I), 1400–2200 h (fraction II), and 2200–0600 h (fraction III), were collected from four subjects during all experimental days. The third fraction from 2200 h to 0600 h was referred to as night urine.

Determinations of hematocrit, serum total protein, osmolality, electrolytes, and hormones. Hematocrit was measured by the centrifuge method using a capillary tube. The serum concentration of total protein was estimated by the biuret method. Osmolality was assessed by the freezing-point method, sodium (Na) and potassium (K) concentrations by the flame photometry, chloride (Cl) by the conductometric titration method, and creatinine (Cr) by the Folin-Wu method.

Hormones were determined by radioimmunoassay (RIA). Plasma renin activity (PRA) and aldosterone were measured using kits purchased from Dainabot RI Laboratory (Tokyo). ANP was measured as follows. Blood samples were obtained into a chilled polypropylene tube containing Na_2EDTA (1 mg/ml) and aprotinin (500 kIU/ml). After centrifugation, 2 ml of plasma was applied to a Sep-Pak C_{18} cartridge, an octadecylsilane column (Waters Ltd., Japan), which was pretreated with 5 ml of methanol, 5 ml of 8 M urea, and 10 ml of distilled water. After washing with 10 ml of distilled water and 10 ml of 0.6% acetic acid, ANP was eluted with 1.5 ml of 50% acetonitrile in 10 mM trifluoroacetic acid (LaRochelle *et al.*, 1980; Miyata *et al.*, 1985). The eluate was dried up under nitrogen gas stream. After reconstitution with assay buffer, samples were stored at $-20°C$ until assay. The assay buffer consisted of 50 mM sodium phosphate (pH 7.0), 0.2% bovine serum albumin, 0.1% Triton X-100, 0.01% NaN_3, 10 mM Na_2EDTA, 0.1 mM N-ethylmaleimide, and aprotinin (500 kIU/ml). This solution was used to dissolve all reagents. Synthetic human α-ANP (1–28) and antiserum against human α-ANP were purchased from Peptide Inst. (Osaka). Human α-ANP was labeled with ^{125}I using IODO-GEN (Pierce Co. Rockford), a derivative of chloramine T (Markwell and Fox, 1978). ^{125}I-labeled ANP was separated from free iodide by use of a Sep-Pak C_{18} cartridge. The RIA incubation mixture consisted of 300 μl of standard α-ANP or sample, 50 μl of antiserum diluted finally to 1 : 30,000 and 50 μl of ^{125}I-α-ANP (approximately 5,000 cpm). The mixture was incubated for 48 h at 4°C in a polypropylene tube. Bound and free ligands were separated by the double-antibody precipitation technique followed by adding 25% polyethylene glycol (MW 6,000) in boric acid-borate buffer (pH 8.6). Radioactivity of the bound fraction was counted in an automatic gamma counter. The amount of ANP that can be detected by this RIA is between 6 and 1,500 pg/tube (Fig. 2). This is within the range that is usually expected for plasma levels of the hormone. The intra- and interassay coefficients of variation were 9 and 16%, respectively. The average recovery of synthetic α-ANP from plasma was 97%. Plasma ADH was simultaneously extracted with ANP using a Sep-Pak C_{18} cartridge. Standard ADH was purchased from Sigma (St. Louis) and antiserum for ADH was from Calbiochem (La Jolla). The procedure of iodination and RIA for plasma ADH was the same

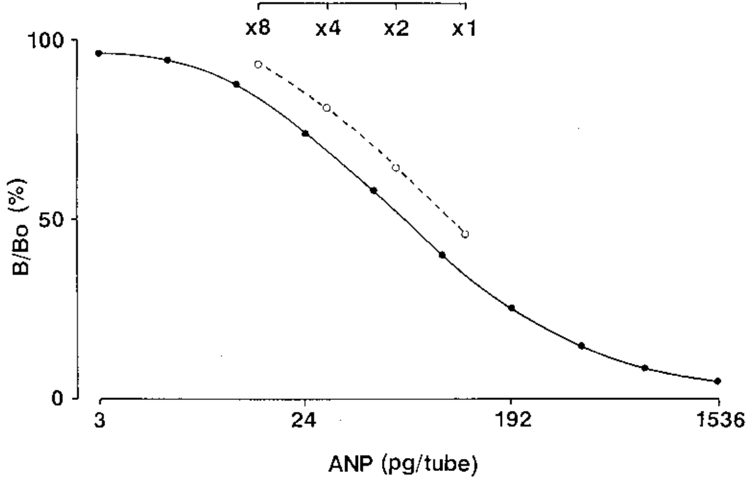

Fig. 2. Standard curve of ANP (solid line) and dilution curve of urine extract (dashed line). The dilution curve of urine extract was parallel to the standard cuve.

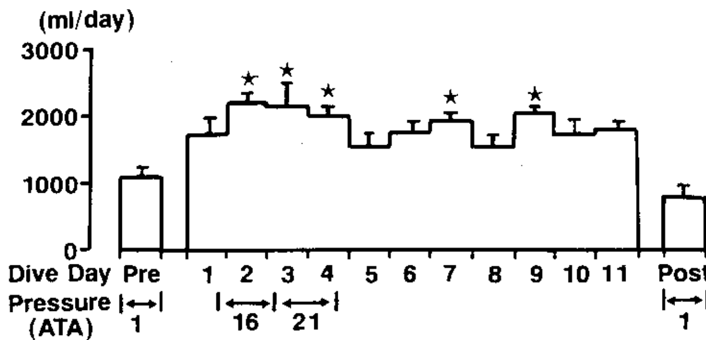

Fig. 3. Changes of 24-h urine flow. *$p<0.05$ vs. pre value.

as in the case of plasma ANP. Urine samples for ANP and ADH were added 1% volume of 6 N HCl and were centrifuged. After neutralization with NaOH, the supernatant was applied to a Sep-Pak C_{18} cartridge for simultaneous extraction of ANP and ADH. The procedure of the RIA was the same as stated above. The dilution curve for urinary ANP was parallel to the standard curve (Fig. 2). Recoveries of ANP and ADH from urine were more than 90%.

Statistical analysis. The data were expressed as mean ± S.E. Statistical analysis was performed with ANOVA (analysis of variance) and Tukey's multiple comparison procedures using SAS (statistical analysis system). In the analysis of data from urine, the experimental days were allocated into four periods as shown in Figs. 4, 5, and 7 in order to simplify the comparison and expression. They are Control (pre- and postdive 1 ATA days), HP 1 (the first high pressure period below

16 ATA consisting of dive Days 1 and 2), HP 2 (the second high pressure period above 16 ATA consisting of dive Days 3, 4, and 5), and Decomp (early decopression period consisting of dive Days 6, 7 and 8). One exception is the analysis of "changes of 24-h urine flow" as shown in Fig. 3. In this case, day-to-day change of urine flow was expressed and analyzed to show the profile of hyperbaric diuresis clearly.

RESULTS

Urine flow

As shown in Fig. 3, 24-h urine flow significantly and consistently increased under hyperbaria including decompression period. It reached approximately 2-fold of the pre-exposure value at 16–21 ATA and returned to the pre-exposure value after decompression.

Eight-hour timed urine flow also tended to increase in each fraction under hyperbaria. The increase was most marked in the night urine. This nocturnal diuresis was observed from the beginning of compression to the early decompression period. At 16–21 ATA, night urine flow not only reached more than 3-fold of that in Control but also exceeded the urine flow of the fractions I or II in contrast with the diurnal pattern in Control (Fig. 4).

Total protein, hematocrit, osmolality, and electrolytes in blood

As shown in Table 1, serum total protein concentration rose significantly and hematocrit tended to rise in the early morning by the exposure to hyperbaria. They were highest at 21 ATA and declined to the pre-exposure value after decompression.

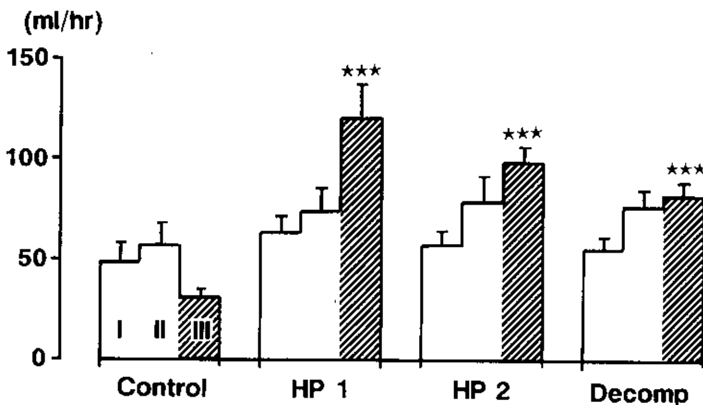

Fig. 4. Changes of 8-h timed urine flow. I, II, and III represent urine fractions I, II, and III, respectively. Shaded bars indicate night urine. Control, pre- and postdive 1 ATA days; HP 1, the first high pressure period below 16 ATA consisting of dive Days 1 and 2; HP 2, the second high pressure period above 16 ATA consisting of dive Days 3–5; Decomp, early decompression period consisting of dive Days 6–8. $^{***}p<0.0001$ vs. each Control value.

Table 1. Changes of serum total protein concentration (TP), hematocrit, serum osmolality, serum electrolytes, and creatinine (Cr) clearance.

Pressure (ATA)	1	16	16	21	1
Dive day	Pre	2	3	4	Post
TP (g/dl)	6.58±0.26	6.72±0.25	7.00±0.20	7.36*±0.30	6.78±0.17
Hematocrit (%)	41.9±0.8	41.8±0.8	42.3±0.7	44.7±1.0	40.0±0.4
Osmolality (mOsm/kg)	289.0±1.5	287.6±1.4	286.4±1.2	279.2±10.4	289.0±2.5
Na (mEq/*l*)	143.6±0.7	142.2±0.6	142.4±0.7	139.6±4.7	143.4±0.7
K (mEq/*l*)	4.24±0.09	4.32±0.04	4.42±0.09	4.20±0.15	3.94±0.07
Cl (mEq/*l*)	107.3±1.0	106.5±0.7	104.6±0.5	100.6*±2.9	106.5±0.6
Cr clearance (*l*/day)	192.6±24.9	191.6±25.1	181.5±21.9	162.2±34.8	179.1±31.1

* $p<0.05$ vs. each pre value.

The levels of serum osmolality, Na and K did not change significantly under hyperbaria while serum Cl fell at 21 ATA.

Osmolality, Na and K excretion, total excretion of osmotic substances in urine, and Cr clearance

Urine osmolality for 24 h fell consistently throughout hyperbaria. This tendency was most marked in the fraction I. In the fraction III, night urine, it also fell markedly at 16–21 ATA and in the early decompression period (Fig. 5). Urinary excretion of Na for 24 h significantly increased in the beginning of compression. Na excretion in the night urine markedly increased at 16–21 ATA and in the early decompression period. It reached more than 2-fold of the pre-exposure value at

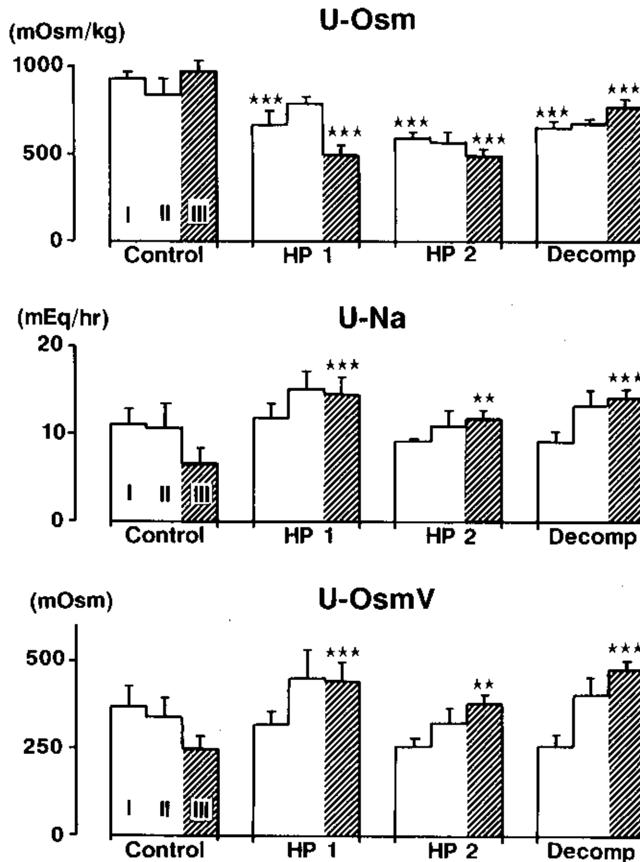

Fig. 5. Changes of osmolality (U-Osm), sodium excretion (U-Na), and calculated total excretion of osmotic substances (U-OsmV) in urine. I, II, and III represent urine fractions I, II, and III, respectively. Shaded bars indicate night urine. $^{**}p<0.005$, $^{***}p<0.0001$ vs. each Control value. See the legend of Fig. 4 about Control, HP 1, HP 2, and Decomp.

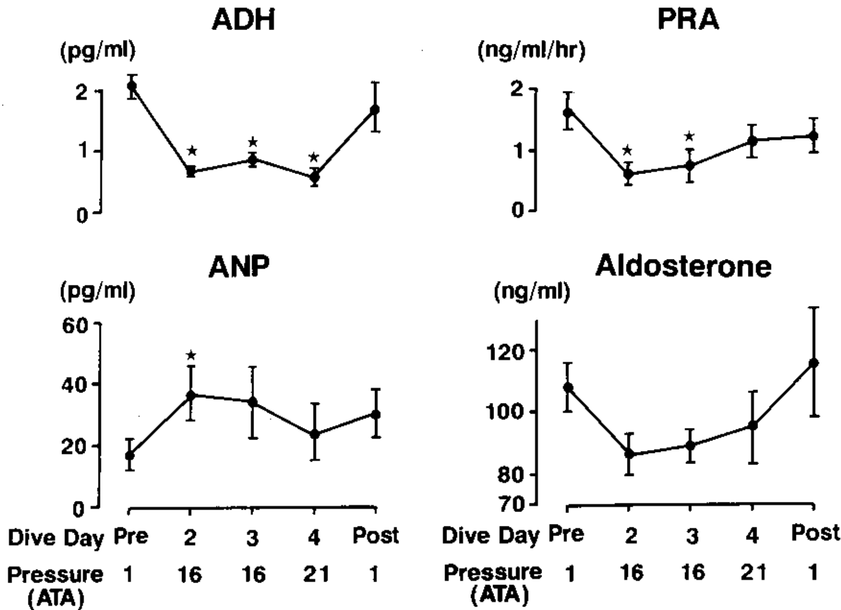

Fig. 6. Changes in plasma ADH, ANP, PRA, and serum aldosterone levels. * $p<0.05$, vs. each pre value.

16–21 ATA. However, Na excretions in both fractions I and II did not change significantly (Fig. 5). Through urinary excretion of K of 24 h did not change significantly, it increased only in the night urine at 16–21 ATA. Na/K ratio in urine rose in the night urine merely in the course of decompression. Calculated total excretion of osmotic substances (U-OsmV) is also shown in Fig. 5. U-OsmV markedly increased only at night under hyperbaria. These substances include Cl, calcium, phosphate, magnesium, urea nitrogen, and uric acid in addition to Na and K. Cr clearance was constant throughout the experimental period (Table 1).

Hormones

Changes in blood hormone levels are shown in Fig. 6. ADH was consistently suppressed during hyperbaria. On the contrary, ANP rose significantly by the exposure to hyperbaria. PRA was suppressed and aldosterone tended to be suppressed by the exposure.

Urinary excretion of hormones is shown in Fig. 7. Excretion of ADH for 24 h tended to be suppressed throughout hyperbaria. This tendency was most remarkable in the fraction I. ADH excretion at night was also suppressed in HP 2. Excretion of ANP for 24 h showed no significant changes. However, ANP excretion at night significantly increased both in HP 1 and in Decomp. Aldosterone excretion in 24h urine and in the fraction I tended to decrease under hyperbaria.

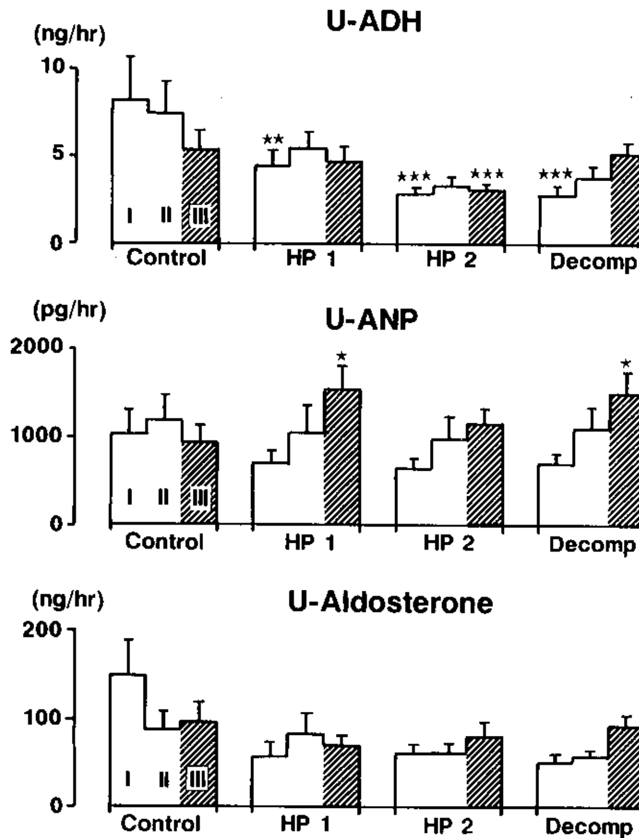

Fig. 7. Changes of urinary ADH, ANP, and aldosterone excretions. I, II, and III represent urine fractions I, II, and III, respectively. Shaded bars indicate night urine. $*p<0.015$, $**p<0.005$, $***p<0.0001$ vs. each Control value. See the legend of Fig. 4 about Control, HP 1, HP 2, and Decomp.

DISCUSSION

A typical hyperbaric diuresis was observed in this experiment at 16–21 ATA in the presence of constant Cr clearance. Increase in urine flow for 24 h under hyperbaria was largely attributable to a marked increase in night urine flow. This nocturnal diuresis was noticed previously at pressures of 31 ATA (NAKAYAMA *et al.*, 1981; SHIRAKI *et al.*, 1984, 1987) and 4 ATA (TAMURA *et al.*, 1983). Therefore, the nocturnal diuresis is thought to be a common phenomenon in saturation dives at above 4 ATA. Urine osmolality decreased both in the daytime and during night while U-OsmV increased only during night. These basic characteristics of the diuresis are qualitatively similar to those observed in Seadragon VI, where analyses in urine samples were performed laying emphasis on the comparison between daytime and

night (NAKAYAMA *et al.*, 1987).

In Seadragon VI, plasma ADH level fell and urinary excretion of ADH decreased in the daytime (0700–1900 h) at high pressure. It was implied that the suppressed secretion of ADH contributed to water diuresis especially in the daytime (CLAYBAUGH *et al.*, 1987; SHIRAKI *et al.*, 1987). In our experiment, plasma ADH in the morning fell significantly during hyperbaria and ADH excretion in the fraction I decreased under hyperbaria. Linear regression analysis showed a positive correlation between ADH excretion and urine osmolality in the fraction I ($r=0.87$, $p<0.005$). These observations confirm the involvement of ADH in the daytime diuresis under hyperbaria. Though suppressed ADH excretion was also observed even during night in Seadragon VI as well as in our experiment, it is not sufficient for full explanation of the mechanism of nocturnal diuresis at high pressure mainly because of an aspect of osmotic diuresis in the night urine (SHIRAKI *et al.*, 1987). Moreover, ADH excretion did not always decreases at night under hyperbaria as seen in Seadragon IV (CLAYBAUGH *et al.*, 1984) despite a marked increase in night urine flow. Therefore, some other factors should be considered to clarify the mechanism of the nocturnal diuresis under hyperbaria, regardless of whether or not ADH plays any role in it.

There are three reports on dive experiments where plasma ANP levels were estimated (MOON *et al.*, 1987; NIU *et al.*, 1990; SAGAWA *et al.*, 1990). In the dive at 2.5 ATA carried out by NIU *et al.* (1990), diuresis did not occur and plasma ANP remained unchanged. MOON *et al.* (1987) suggested possible involvement of ANP in hyperbaric diuresis. SAGAWA *et al.* (1990) did not observe a rise in plasma ANP levels in spite of sustained diuresis. They attributed the result to the time of the day and frequency of blood sampling. However, frequent blood samplings when sleeping are not easy in actuality in a hyperbaric environment. We measured ANP levels not only in blood in the morning but also in urine including nighttime in the current experiment. Plasma ANP rose significantly on the dive Day 2 at 16 ATA. We obtained blood samples at 0600–0630 h while SAGAWA *et al.* (1990) did at 0700–0730 h. The earlier timing of sampling might lead us to observation of a rise in plasma ANP. Though a RIA of ANP in human urine was reported by MARUMO *et al.* (1986), physiological significance of urinary immunoreactive ANP has not been established yet. As shown in Fig. 2, the dilution curve for urinary immunoreactive ANP was parallel to the standard curve. We also observed that the position of the immunoreactivity in the eluate was identical to that of authentic ANP in a preliminary gel-filtration experiment using Sephadex G-50 (Pharmacia, Uppsala). Moreover, we confirmed that an increase of urine flow occurred after a rise of plasma ANP level followed by an increase of urinary ANP excretion in an *in vivo* study where human subjects were immersed into water to the neck (unpublished observation). In the current dive, ANP excretion increased only in the night urine at high pressure. Interestingly, the changing pattern of urinary ANP resembled those of urine flow, Na excretion and U-OsmV in the night urine. Indeed, ANP excretion positively correlated with urine flow, Na excretion, and U-OsmV

in the night urine ($r=0.62$, $p<0.05$; $r=0.75$, $p<0.005$; $r=0.63$, $p<0.005$, respectively). This indicates an important role of ANP in the nocturnal diuresis which composes a major part of hyperbaric diuresis.

PRA fell and serum aldosterone level tended to fall in the morning by the exposure to hyperbaria in the current study. The results are in concordance with those of plasma ADH and ANP. Urinary excretion of aldosterone also tended to decrease. However, these results conflict with those in the previous observations. PRA rose and aldosterone secretion increased in Seadragon IV and VI (CLAYBAUGH *et al.*, 1984, 1987). This may be attributable, in part, to oxygen partial pressure during hyperbaria which was higher in our experiment than in other experiments. WALKER *et al.* (1981) reported that hyperoxia suppressed renin release in the dog. According to WEIHL (1978), optimum oxygen partial pressure at high pressure is between 0.3 and 0.4 ATA. Higher O_2 pressure of 0.42–0.43 ATA during hyperbaria in our experiment than 0.3 ATA in Seadragon VI and 0.4 ATA in Seadragon VI may relate to suppression of renin-aldosterone system. In any case, hyperbaric diuresis occurs regardless of stimulation or suppression of renin-aldosterone system. This suggests that renin and aldosterone do not play major roles in hyperbaric diuresis but react secondarily.

From these results, we assume a possible mechanism of hyperbaric diuresis as follows. Firstly, high gas density in the chamber causes suppression of insensible water loss and augments negative pressure breathing, resulting in an increase of venous return to the heart. Secondly, subtle cold stress should be taken into consideration. Though attempts have been made to maintain a so-called thermoneutral condition in recent dive experiments (CLAYBAUGH *et al.*, 1984; SHIRAKI *et al.*, 1984, 1985), slight changes in chamber temperature might be inevitable. Since heat conductivity of helium gas is very high, cold stress may be easily caused (NAKAYAMA *et al.*, 1981). In fact, it was demonstrated that the secretion of thermogenic hormones such as thyroid hormones increased in hyperbaric heliox environments (MATSUI, 1983). This stress causes peripheral vasoconstriction, also resulting in an increase of venous return. Thirdly, being supine at night should be considered as an important factor causing increase of venous return (NAKAYAMA *et al.*, 1981; SHIRAKI *et al.*, 1984, 1985). All these factors increase intra-thoracic blood volume and stretch the atrial wall. Consequently, ADH secretion is suppressed and ANP secretion is stimulated. Both these changes cause hyperbaric diuresis.

Thus, it is suggested that two hormones which have effects opposite to each other in water balance contribute to hyperbaric diuresis. However, the reason why ADH secretion was suppressed both in the daytime and during night while ANP secretion was stimulated preferentially during night is not clear since similar conditions should suppress ADH secretion and stimulate ANP secretion simultaneously. It may be speculated that ADH and ANP have different thresholds in their secretions. In other words, more augmented changes in the physiological condition may be required in stimulation of ANP secretion than in suppression of ADH secretion. Further investigation should be carried out.

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REFERENCES

ALEXANDER, W. C., LEACH, C. S., FISCHER, C. L., LAMBERTSEN, C. J., and JOHNSON, P. C. (1973) Hematological, biochemical, and immunological studies during a 14-day continuous exposure to 5.2% O_2 in N_2 at pressure equivalent to 100 FSW (4 ata). *Aerosp. Med.*, **44**: 850–854.

BENNETT, P. B. and GRAY, S. P. (1971) Changes in human urine and blood chemistry during a simulated oxygen-helium dive to 1,500 feet. *Aerosp. Med.*, **42**: 868–874.

CANTIN, M. and GENEST, J. (1985) The heart and the atrial natriuretic factor. *Endocrine Rev.*, **6**: 107–127.

CLAYBAUGH, J. R., HONG, S. K., MATSUI, N., NAKAYAMA, H., PARK, Y. S., and MATSUDA, M. (1984) Responses of salt- and water-regulating hormones during a saturation dive to 31 ATA (SEADRAGON IV). *Undersea Biomed. Res.*, **11**: 65–80.

CLAYBAUGH, J. R., MATSUI, N., HONG, S. K., PARK, Y. S., NAKAYAMA, H., and SHIRAKI, K. (1987) Seadragon VI: A 7-day dry saturation dive at 31 ATA III. Alterations in basal and circadian endocrinology. *Undersea Biomed. Res.*, **14**: 401–411.

DE BOLD, A. J., BORENSTEIN, H. B., VERESS, A. T., and SONNENBERG, H. (1981) A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci.*, **28**: 89–94.

HONG, S. K. (1975) Body fluid balance during saturation diving. *In*: International Symposium on Man in the Sea, ed. by HONG, S. K., Undersea Medical Society, Bethesda, pp. 127–143.

HONG, S. K., CLAYBAUGH, J. R., FRATTARI, V., JOHNSON, R., KURATA, F., MATSUDA, M., MCDONOUGH, A. A., PAGANELLI, C. V., SMITH, R. M., and WEBB, P. (1977) Hana Kai II: A 17-day dry saturation dive at 18.6 ATA. III. Body fluid balance. *Undersea Biomed. Res.*, **4**: 247–265.

HONG, S. K., CLAYBAUGH, J. R., and SHIRAKI, K. (1983) Body fluid balance in the high pressure environment. *In*: Hyperbaric Medicine and Underwater Physiology, ed. by SHIRAKI, K. and MATSUOKA, S., The Program Committee of III UOEH Symposium, Kitakyushu, pp. 223–234.

LAROCHELLE, F. T., Jr., NORTH, W. G., and STERN, P. (1980) A new extraction of arginine vasopressin from blood: The use of octadecasilyl-Silica. *Pflügers Arch.*, **387**: 79–81.

LEACH, C. S., ALEXANDER, W. C., FISCHER, C. L., LAMBERTSEN, C. J., and JOHNSON, P. C. (1973) Endocrine studies during a 14-day continuous exposure to 5.2% O_2 in N_2 at pressure equivalent to 100 fsw (4 ATA). *Aerosp. Med.*, **44**: 855–859.

MARKWELL, M. A. K. and FOX, C. F. (1978) Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3,6-diphenylglycoluril. *Biochemistry*, **17**: 4807–4817.

MARUMO, F., SAKAMOTO, H., ANDO, K., ISHIGAMI, T., and KAWAKAMI, M. (1986) A highly sensitive radioimmunoassay of atrial natriuretic peptide (ANP) in human plasma and urine. *Biochem. Biophys. Res. Commun.*, **137**: 231–236.

MATSUDA, M., NAKAYAMA, H., ARITA, H., MORLOCK, J. F., CLAYBAUGH, J. R., SMITH, R. M., and HONG, S. K. (1978) Physiological responses to head-out immersion in water at 11 ATA. *Undersea Biomed. Res.*, **5**: 37–52.

MATSUDA, M., NAKAYAMA, H., KURATA, F., CLAYBAUGH, J. R., and HONG, S. K.

(1975) Physiology of man during a 10-day dry heliox saturation dive (SEATOPIA) to 7 ATA. II. Urinary water, electrolytes, ADH, and aldosterone. *Undersea Biomed. Res.*, **2**: 119–131.

MATSUI, N. (1983) Hormonal responses to hyperbaric heliox environment in man. *In*: Hyperbaric Medicine and Underwater Physiology, ed. by SHIRAKI, K. and MATSUOKA, S., The Program Committee of III UOEH Symposium, Kitakyusyu, pp. 213–222.

MIYATA, A., KANGAWA, K., TOSHIMORI, T., HATOH, T., and MATSUO, H. (1985) Molecular forms of atrial natriuretic polypeptides in mammalian tissues and plasma. *Biochem. Biophys. Res. Commun.*, **129**: 248–255.

MOON, R. E., CAMPORESI, E. M., XUAN, T., HOLTHAUS, J., MITCHELL, P. R., and WATKINS, W. D. (1987) ANP and diuresis during compression to 450 and 600 MSW. *Undersea Biomed. Res.* (Suppl.), **14**: 43–44.

NAKAYAMA, H., HONG, S. K., CLAYBAUGH, J. R., MATSUI, N., PARK, Y. S., OHTA, Y., SHIRAKI, L., and MATSUDA, M. (1981) Energy and body fluid balance during a 14-day dry saturation dive at 31 ATA (SEADRAGON IV). *In*: Underwater Physiology VII, ed. by BACHRACH, A. J. and MATZEN, M., Undersea Medical Society, Bethesda, pp. 541–554.

NAKAYAMA, H., MURAI, T., and HONG, S. K. (1987) Seadragon VI: A 7-day dry saturation dive at 31 ATA I. Objectives, design, and scope. *Undersea Biomed. Res.*, **14**: 377–385.

NEUMAN, T. S., GOAD, R. F., HALL, D., SMITH, R. M., CLAYBAUGH, J. R., and HONG, S. K. (1979) Urinary excretion of water and electrolytes during open-sea saturation diving to 850 fsw. *Undersea Biomed. Res.*, **6**: 291–302.

NIU, A. K. C., HONG, S. K., CLAYBAUGH, G. H., GOLDINGER, J. M., KWON, O., LI, M., RANDALL, E., and LUNDGREN, C. E. G. (1990) Absence of diuresis during a 7-day saturation dive at 2.5 ATA N_2-O_2. *Undersea Biomed. Res.*, **17**: 189–199.

RAYMOND, L. W., RAYMOND, N. S., FRATTALI, V. P., SODE, J., LEACH, C. S., and SPAUR, W. H. (1980) Is the weight loss of hyperbaric habitation a disorder of osmoregulation? *Aviat. Space Environ. Med.*, **51**: 397–401.

SAGAWA, S., CLAYBAUGH, J. R., SHIRAKI, K., PARK, Y. S., MOHRI, M., and HONG, S. K. (1990) Characteristics of increased urine flow during a dry saturation dive at 31 ATA. *Undersea Biomed. Res.*, **17**: 13–22.

SCHAFFER, K. E., CAREY, C. R., and DOUGHERTY, J., Jr. (1970) Pulmonary gas exchange and urinary electrolyte excretion during saturation-excursion diving to pressures equivalent to 800 and 1000 feet of seawater. *Aerosp. Med.*, **41**: 856–864.

SHIRAKI, K., HONG, S. K., PARK, Y. S., SAGAWA, S., KONDA, N., CLAYBAUGH, J. R., TAKEUCHI, H., MATSUI, N., and NAKAYAMA, H. (1987) Seadragon VI: A 7-day dry saturation dive at 31 ATA II. Characteristics of diuresis and nocturia. *Undersea Biomed. Res.*, **14**: 387–400.

SHIRAKI, K., SAGAWA, S., KONDA, N., and HONG, S. K. (1985) Hyperbaric diuresis and nocturia—A review. *J. UOEH.*, **7**: 61–72.

SHIRAKI, K., SAGAWA, S., KONDA, N., NAKAYAMA, H., and MATSUDA, M. (1984) Hyperbaric diuresis at a thermoneutral 31 ATA He-O_2 environment. *Undersea Biomed. Res.*, **11**: 341–353.

TAMURA, K., OKAZAKI, S., HATANO, T., OGAWA, K., SUEDA, K., YAMAMOTO, M., SEO, H., MATSUI, N., KANDA, K., TAKEUCHI, H., and SEKI, K. (1983) Hormone changes in man during saturation exposure at 4 ATA helium-oxygen atmosphere (SM-I). *Environ. Med.*, **27**: 17–28.

WALKER, B. R., HONG, S. K., MOOKERJEE, B. K., and KRASNEY, J. A. (1981) Suppressed

renin release during hyperoxia in the conscious dog. *Undersea Biomed. Res.*, **8**: 137–145.

WEIHL, A. C. (1978) Endocrine responses to elevated ambient pressure. *In*: Environmental Endocrinology, ed. by ASSENMACHER I. and FARNER, D. S., Springer-Verlag, Berlin, pp. 276–282.

WRIGHT, W. B., FISCHER, A. B., HENDRICKS, P. L., BRODY, J. S., and LAMBERTSEN, C. J. (1973) Pulmonary function studies during a 14-day continuous exposure to 5.3% O_2 in N_2 at pressure equivalent to 100 FSW (4 ata). *Aerosp. Med.*, **44**: 837–843.