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Urinary ANP, ADH, and electrolyte excretion during saturation-excursion diving to pressures equivalent to 250 and 300 m

H. Y. TAO, H. J. CHEN, H. ZHANG, J. GUO, and F. K. RONG

Department of Diving Physiology, Faculty of Naval Medicine, Second Military Medical University, Shanghai 200433, China; and Diving Medicine Center, Shanghai Salvage Company, Shanghai 200090, China

Tao HY, Chen HJ, Zhang H, Guo J, Rong FK. Urinary ANP, ADH, and electrolyte excretion during saturation-excursion diving to pressures equivalent to 250 and 300 m. Undersea Biomed Res 1992; 19(3):159–169.—Four male divers were exposed to an environment of 26 and 31 atm abs He-O_2 for 2 days. Urine was collected during the day (0700–1900 h) and at night (1900–0700 h) before (predive 1 atm abs air), during, and after (decompression and postdive 1 atm abs air) the exposure. Urine flow increased markedly and was mostly attributable to the urine flow during 1900–0700 h. The secretion of atrial natriuretic polypeptide (ANP) increased only at night during hyperbaria. On the other hand, the secretion of antidiuretic hormone (ADH) was suppressed, but increased during compression and the early phase of hyperbaric exposure before it decreased. Linear regression analysis showed that urinary excretion of ANP was correlated significantly with urine flow ($r = 0.88$, $P < 0.01$) and that excretion of ADH negatively correlated with urine flow ($r = -0.61$, $P < 0.01$). Urinary excretion of Na, Cl, Ca, and Mg increased significantly at night during hyperbaria, whereas there was no consistent change in the excretion of K and P. These results suggest that both stimulated ANP secretion and suppressed ADH secretion correlate with the increase of urine and that nocturia is mostly attributable to stimulated ANP secretion. We observed that urinary excretion of ANP increased significantly as early as during the compression phase, which suggested that ANP plays a decisive role in the early diuresis.

hyperbaria	atrial natriuretic polypeptide
diuresis	antidiuretic hormone
nocturia	electrolytes

A hyperbaric environment causes various metabolic alterations in men, including increased urine flow, called hyperbaric diuresis (1). This phenomenon was first reported by Hamilton (2) and was confirmed in many subsequent dives (3–5). Although the mechanism of the diuresis is not fully understood, several factors have been proposed as causes. They include cold stress (2), osmotic gas gradients (6), and augmented negative pressure breathing (7–8). Hong et al. (8, 9) presumed that suppression of insensible water loss was the primary cause for the diuresis. These conditions all lead to an increase of intrathoracic blood volume, resulting in suppres-

sion of antidiuretic hormone (ADH) secretion and subsequent water diuresis. The important role of ADH in hyperbaric diuresis has been pointed out by various investigators (9–12). However, hyperbaric diuresis is often accompanied by an increase of some electrolytes (e.g., Na, K, Ca, and P). One unexplained finding is that the urinary excretion of ADH always increases during the early phase of hyperbaric exposure before it decreases, and the reduction in ADH excretion is still maintained during the postdive period when the hyperbaric diuresis is no longer present (13). These observations suggested that some factor(s) other than ADH, such as "third factor" or "natriuretic factor," might be involved (8, 12–13). To further investigate the mechanism of the diuresis, we observed the changes of urine flow, urinary excretion of atrial natriuretic polypeptide (ANP), ADH, and some electrolytes during saturation-excursion diving to 26 and 31 atm abs in 4 men, and more closely examined the role of ANP in hyperbaric diuresis.

SUBJECTS AND METHODS

Subjects

Four male divers were selected on the basis of rigorous physical and psychologic examinations. On the average, they were 26 (21–31) yr old, 171.3 (167–175) cm in height, and 68.3 (65–72) kg in weight. All subjects were well-trained professional divers and were involved in earlier saturation dives (N_2–O_2) carried out at the Shanghai Salvage Company.

Dive profile

The experiment was carried out in a hyperbaric chamber at Shanghai Salvage Company. The experimental protocol is shown in Fig. 1. Before compression, all the

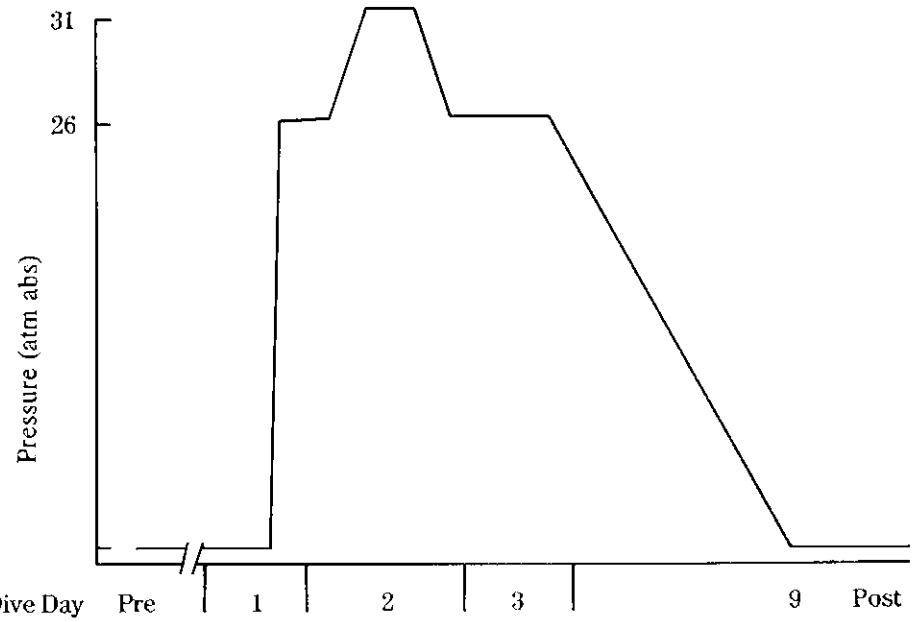

Fig. 1. Dive profile.

subjects entered the hyperbaric chamber for the predive 1 atm abs control period (Pre). Compression started at 0855 h on 1 December 1990 (Dive Day 1) with He-O_2 (18% O_2) gas until 2.1 atm abs and pure helium thereafter and it was carried out at rates of 6 atm abs 1 h (from 1 to 2.1 atm abs), 18 atm abs/h (from 2.1 to 16 atm abs), 6 atm abs/h (from 16 to 21 atm abs) and 1 atm abs/h (from 21 to 26 atm abs). After a stop at 26 atm abs from 1651 h on Dive Day 1 to 1649 h on Dive Day 2, all of the subjects made an excursion dive to 31 atm abs and came back to saturation depth (26 atm abs) at 1734 h. Decompression started at 1810 h on Dive Day 3 at rates of 30 min/m (from 26 to 16 atm abs), 35 min/m (from 16 to 11 atm abs), 40 min/m (from 11 to 6 atm abs), 45 min/m (from 6 to 2.5 atm abs), 50 min/m (from 2.5 to 2 atm abs), and 60 min/m (from 2 to 1 atm abs). Day 8 after the end of decompression was designated as the postdive 1 atm abs control period (Post).

Environmental parameters

The ambient gas was maintained at 0.38–0.42 atm abs O_2 (before saturation decompression), at 0.60–0.63 atm abs O_2 (during decompression phase), and at 0.30–0.79 atm abs N_2. CO_2 pressure was kept at <0.006 atm abs. Chamber temperature was controlled at 27°C at 1 atm abs and 28°–32°C at high pressure. Relative humidity was maintained at 50–70%.

Urine collection

To examine the difference in character of the urine between day and night, 12-h urine samples, 0700–1900 and 1900–0700 h, were collected from the 4 subjects during all experimental days. Urine samples from 0700–1900 h were referred to as day urine and those from 1900–0700 h as night urine. Volumes were recorded, and 1 *N* HAc was immediately added to adjust pH to 4.5. Containers were locked out at 0700 and 1900 h every day.

Determinations of ANP and ADH

Urine samples were heated at 85°C for 15 min and then centrifuged at 3000 rpm for 15 min. Ten or 20 ml of supernatant was applied to a Sep-Pak C_{18} cartridge (Waters Associates, USA), which was pretreated with 5 ml of 100% acetonitrile and 20 ml of ion-free water. After washing with 20 ml of 4% acetic acid, ANP and ADH were eluted with 5 ml of 75% acetonitrile. The eluate was dried up under a nitrogen gas stream. After reconstitution with assay buffer, samples were stored at −40°C until assay. Urinary ANP was determined by a radioimmunoassay (RIA) kit (Research Institute kit for RIA technique, Tong Ji University, Shanghai), and urinary ADH was also determined by an RIA kit (Department of Neurobiology, Second Military Medical University, Shanghai). The detection limit for ADH and ANP was, respectively, 2 and 10 pg/tube with a recovery of 96 and 92%. In a power-point system (14), 32 and 29 points were obtained, respectively, indicating a good quality for the RIAs.

Determinations of electrolytes (15)

Na and K concentrations were assessed by flame photometry, Cl by an electrode, Ca by titration with EDTA-Na_2, Mg by a methylthymol blue-ethanamide method, and P by colorimetry with ferrous sulfate phosphomolybdic blue (PMB).

Data analysis

The data were expressed as mean ± SE. Statistical analysis was performed with paired t tests and linear regression. A difference was considered significant if $P < 0.05$.

RESULTS

Urine flow

As shown in Figs. 2–4, 24-h urine flow and the night urine consistently increased significantly under hyperbaria, including the decompression period. The night urine reached approximately threefold of the preexposure value during Dive Days 6 and 7 and returned to the preexposure value after decompression. The increase was most marked in the night urine, whereas there was no statistical significance in the day urine.

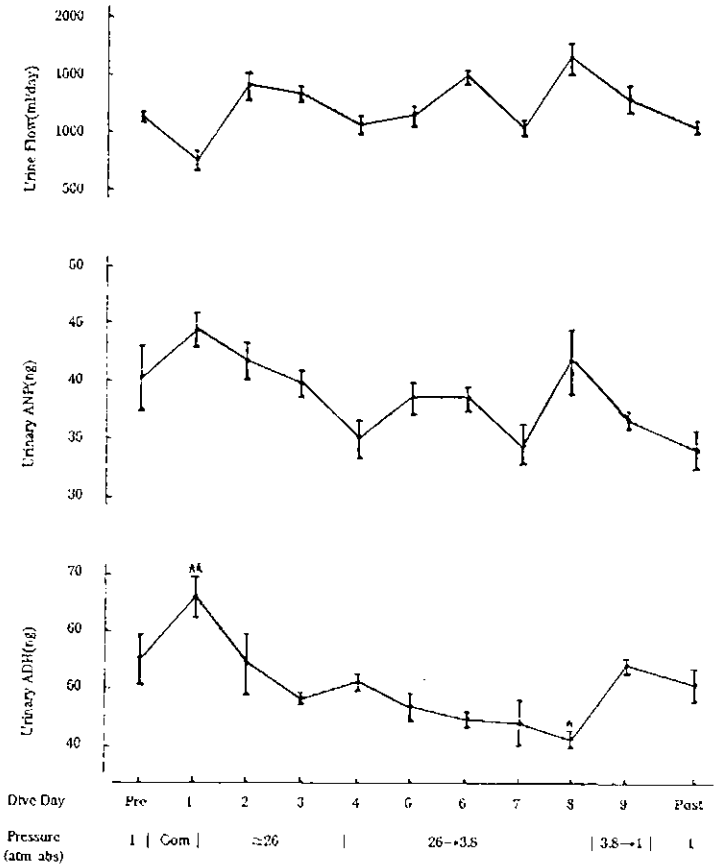

Fig. 2. Urine flow and urinary ANP and ADH (0700–1900 h). *Asterisk* = $P < 0.05$, *double asterisk* = $P < 0.01$ from corresponding predive value. Values are means ± SE of 4 subjects.

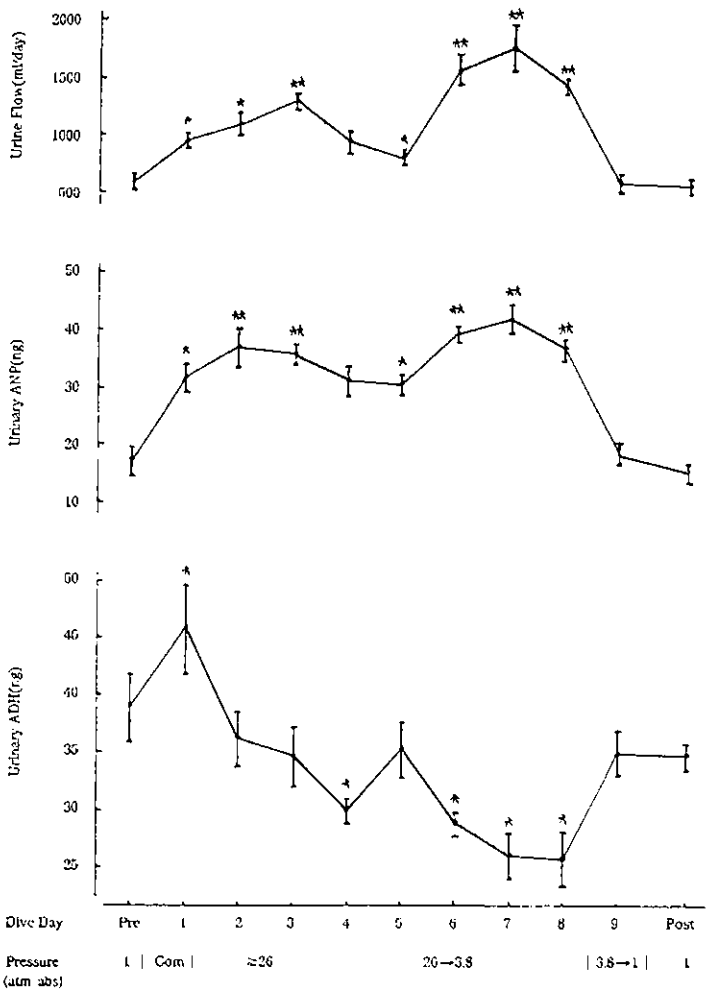

Fig. 3. Urine flow and urinary ANP and ADH (1900–0700 h). *Asterisk* = $P < 0.05$, *double asterisk* = $P < 0.01$ from corresponding predive value. Values are means ± SE of 4 subjects.

Urinary atrial natriuretic polypeptide

Urinary excretion of ANP is shown in Figs. 2–4. The excretion at night increased significantly throughout hyperbaria, except on Dive Day 4. Linear regression analysis showed that urinary ANP excretion correlated significantly with urine flow ($r = 0.88$, $P < 0.01$).

Urinary antidiuretic hormone

Urinary excretion of ADH is shown in Figs. 2–4. The excretion at night tended to be suppressed throughout hyperbaria except during the early phase of hyperbaric exposure. Linear regression analysis showed that urinary ADH excretion was negatively correlated with urine flow ($r = 0.61$, $P < 0.01$).

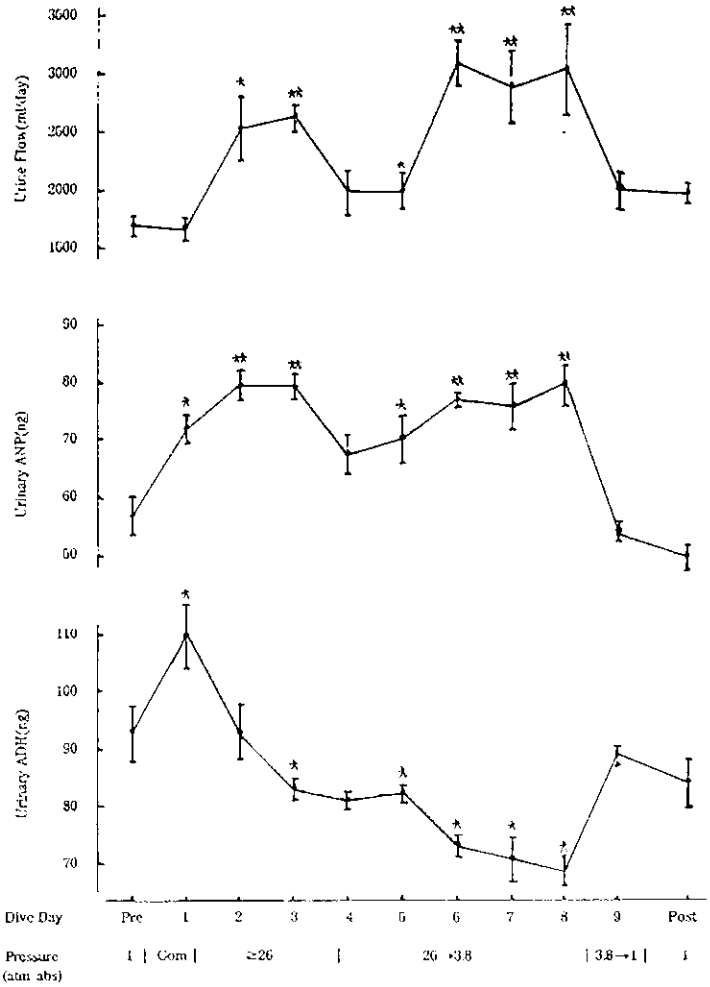

Fig. 4. Urine flow and urinary ANP and ADH (24 h). *Asterisk* = $P < 0.05$, *double asterisk* = $P < 0.01$ from corresponding predive value. Values are means ± SE of 4 subjects.

Urinary electrolytes

As shown in Tables 1–3, urinary excretion of Na, Cl, Ca, and Mg increased markedly at night under hyperbaria. There was no consistent change in the urinary excretion of P and K.

DISCUSSION

A typical hyperbaric diuresis was observed in this experiment, and the increase in daily urine flow at pressure was almost entirely due to the increase in overnight urine flow. This nocturia phenomenon was first observed during the *Seadragon IV* dive (16), and was confirmed in many subsequent dives. The nocturia is thought to be a common phenomenon in saturation dives at above 4 atm abs (1). Statistical evaluation by regression analysis revealed that urinary excretion of ANP was significantly correlated with urine flow, and that excretion of ADH negatively correlated with

TABLE 1
URINARY ELECTROLYTES, 0700–1900 H[a]

Dive Day	K, mmol	Na, mmol	Cl, mmol	Ca, mg	Mg, mg	P, mg
Pre	97.0 ± 10.4	213.7 ± 32.1	171.9 ± 30.2	206.1 ± 35.1	139.5 ± 40.5	498.1 ± 135.0
1	94.3 ± 12.1	117.0 ± 11.2	114.6 ± 9.7	107.1 ± 20.2	48.8 ± 6.7[b]	514.4 ± 109.2
2	102.7 ± 11.1	219.2 ± 32.5	226.7 ± 61.2	190.6 ± 46.6	100.3 ± 12.9	451.7 ± 83.1
3	94.2 ± 9.3	200.1 ± 19.7	206.7 ± 31.4	220.8 ± 30.9	89.1 ± 13.9	387.5 ± 69.6
4	85.4 ± 17.5	172.3 ± 16.4	134.5 ± 25.9	166.4 ± 26.7	82.7 ± 12.5	303.3 ± 23.4[b]
5	67.0 ± 9.0[b]	151.8 ± 13.0[b]	152.4 ± 26.6	143.7 ± 21.0	74.4 ± 16.3	319.4 ± 44.1
6	111.7 ± 16.4	218.0 ± 26.5	219.6 ± 36.0	178.9 ± 39.0	72.7 ± 10.5	441.2 ± 76.4
7	52.2 ± 11.5[b]	156.8 ± 20.5	148.9 ± 39.5	128.5 ± 15.2	48.6 ± 11.5[b]	312.9 ± 65.5
8	54.3 ± 8.1[b]	226.0 ± 40.7	194.0 ± 38.6	151.8 ± 26.1	100.7 ± 21.5	290.3 ± 41.3[b]
9	48.8 ± 4.5[b]	170.3 ± 18.3	98.7 ± 8.7[b]	112.5 ± 8.8[b]	82.0 ± 16.3[b]	329.1 ± 147.1[b]
Post	89.6 ± 7.8	181.3 ± 21.5	148.5 ± 27.1	172.9 ± 28.0	128.9 ± 14.9	335.2 ± 36.4

[a]Values are means ± SE of 4 subjects. [b]$P < 0.05$ from corresponding predive value.

TABLE 2
URINARY ELECTROLYTES, 1900–0700 H[a]

Dive Day	K, mmol	Na, mmol	Cl, mmol	Ca, mg	Mg, mg	P, mg
Pre	53.9 ± 10.1	99.9 ± 14.8	72.7 ± 8.3	86.0 ± 11.1	36.9 ± 6.6	485.3 ± 36.1
1	91.7 ± 12.3[b]	131.1 ± 7.2[b]	161.7 ± 18.8[b]	124.3 ± 16.6[b]	51.5 ± 7.1	668.0 ± 147.4
2	83.2 ± 5.1	166.6 ± 18.9[b]	152.6 ± 17.7[b]	135.9 ± 11.8[b]	66.9 ± 10.7[b]	399.7 ± 26.5[b]
3	96.8 ± 13.2[b]	183.5 ± 24.7[b]	191.4 ± 30.1[b]	200.5 ± 38.7[b]	87.2 ± 11.7[b]	369.7 ± 46.2[b]
4	55.0 ± 12.7	133.3 ± 20.1	108.5 ± 25.8	150.5 ± 30.1	57.7 ± 10.9	290.8 ± 68.9[b]
5	45.4 ± 6.8	102.9 ± 12.1	106.3 ± 12.3	152.0 ± 17.5[b]	42.1 ± 8.1	515.0 ± 103.3
6	72.4 ± 10.3	217.0 ± 24.3[b]	203.8 ± 26.7[b]	175.0 ± 12.1[b]	81.3 ± 9.2[b]	633.7 ± 127.2
7	56.8 ± 7.9	255.1 ± 30.7[b]	243.7 ± 31.9[b]	173.1 ± 23.4[b]	86.2 ± 12.7[b]	450.2 ± 50.4
8	55.3 ± 8.4	176.1 ± 21.2[b]	182.4 ± 19.9[b]	212.7 ± 34.5[b]	52.9 ± 10.1	505.7 ± 125.9
9	30.7 ± 4.6	96.0 ± 8.7	61.8 ± 8.9	90.5 ± 11.3	41.9 ± 4.2	306.7 ± 103.1[b]
Post	48.9 ± 6.9	85.6 ± 9.3	81.3 ± 9.1	80.3 ± 9.5	36.4 ± 4.5	434.4 ± 66.1

[a]Values are means ± SE of 4 subjects. [b]$P < 0.05$ from corresponding predive value.

TABLE 3
URINARY ELECTROLYTES, 24 H[a]

Dive Day	K, mmol	Na, mmol	Cl, mmol	Ca, mg	Mg, mg	P, mg
Pre	150.9 ± 27.1	313.5 ± 38.9	244.5 ± 30.4	292.1 ± 34.5	176.3 ± 29.8	983.2 ± 142.2
1	186.0 ± 29.8	248.0 ± 12.7[b]	276.2 ± 25.0[b]	231.4 ± 31.7	100.3 ± 5.2[b]	1,182.0 ± 248.7
2	186.0 ± 17.8	385.8 ± 48.2[b]	379.2 ± 51.7[b]	326.5 ± 47.8	167.2 ± 12.4	851.5 ± 121.2
3	191.0 ± 28.8[b]	83.6 ± 46.7[b]	398.1 ± 66.8[b]	421.3 ± 88.7	176.3 ± 12.8	757.5 ± 71.8
4	140.4 ± 25.7	305.5 ± 47.4	243.0 ± 50.1	316.8 ± 61.2	140.3 ± 15.2	593.2 ± 71.1[b]
5	112.4 ± 16.1	254.6 ± 21.7	258.7 ± 30.3	295.6 ± 38.1	116.5 ± 14.4[b]	834.2 ± 146.4
6	184.1 ± 25.7	435.0 ± 49.7[b]	423.3 ± 37.0[b]	353.8 ± 51.6	154.0 ± 7.6	1,074.3 ± 171.5
7	108.9 ± 21.1[b]	411.9 ± 61.2[b]	392.7 ± 51.1[b]	301.6 ± 35.2	134.8 ± 23.6	763.3 ± 104.9
8	109.6 ± 14.7[b]	402.1 ± 79.1[b]	376.3 ± 61.7[b]	364.5 ± 48.8	153.6 ± 22.3	795.8 ± 168.3
9	79.5 ± 8.7[b]	266.3 ± 31.7	160.5 ± 28.7	203.5 ± 5.7	123.9 ± 18.8	636.8 ± 211.5
Post	138.5 ± 18.9	266.9 ± 45.8	229.8 ± 38.6	253.2 ± 31.8	165.3 ± 12.6	769.0 ± 107.1

[a]Values are means ± SE of 4 subjects. [b]$P < 0.05$ from corresponding predive value.

urine flow. We observed that the excretion of ADH increased markedly during compression and the early phase of hyperbaric exposure, but urine flow did not decrease or even increase markedly. This clearly indicates that there must be other mechanisms for the early diuresis besides the inhibition of ADH. It is possible that some factors counteracting the action of ADH (e.g., norepinephrine, prostaglandin, ANP) may be activated during the early hyperbaric period. We observed that urinary excretion of ANP increase significantly as early as during the compression phase, which suggested that ANP plays a decisive role in early diuresis. Out of five reports (1, 4, 17–19) of measurements of plasma ANP or/and urinary excretion of ANP on dive experiments, only Sagawa (17) did not observe a rise in plasma ANP levels in spite of sustained diuresis.

In our experiment, the changing pattern of urinary ANP resembled those of urine flow in the night urine. Indeed, ANP excretion correlated positively with urine flow and Na excretion in the night urine. This also indicates an important role of ANP in the nocturia which composes almost the whole hyperbaric diuresis. The reason why ANP excretion was stimulated at high pressure is not clear. We presume that factors causing the increase of urinary ANP are similar to those causing the decrease of urinary ADH. These factors lead to an increase of intrathoracic blood volume, resulting in secreting of ANP from cardiocytes of the atria and suppression of ADH secretion and subsequent diuresis. Indeed, impedance cardiography data indicated an increase at high pressure in thoracic blood volume (20), one of the main factors that stimulates secretion of ANP (21, 22).

Why did urinary excretion of ADH increase during the early phase of hyperbaric exposure? We presume that it might be a temporary response to hyperbaric stress. Urinary excretion of Na, Cl, Ca, and Mg increased markedly in the night urine at high pressure whereas there were no consistent changes in urinary excretion of K and P. Some investigators observed that urinary excretion of Ca and P increased significantly at high pressure, but they could not explain the possible cause for increased Ca and P excretion. Tang et al. (23) observed that i.v. α-hANP in normal volunteers caused an obvious increase of urinary excretion of Ca, Mg, and P as well as an increase in Na and Cl. The increase of urinary excretion of Ca, P, and Mg at high pressure might be the results of stimulated ANP secretion. We conclude that the increase in urine volume at night is a result of the increase of intrathoracic blood volume leading to stimulated ANP, which may play a greater role in the nocturia, especially during the early phase of hyperbaric exposure. However, the effect of ANP on urinary excretion of electrolytes should be investigated more systemically in future dives.

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