

Effect of Head-Out Immersion on Plasma Atrial Natriuretic Factor in Man (42497)

D. R. PENDERGAST, A. J. DE BOLD, M. PAZIK, AND S. K. HONG

Department of Physiology, State University of New York at Buffalo, Buffalo, New York 14214, and Department of Pathology, Queen's University, Kingston, Ontario, Canada

Abstract. This study was conducted to examine the role of atrial natriuretic factor (ANF) in the development of diuresis and natriuresis in response to the head-out immersion in 35°C water. Six male subjects were hydrated (0.5% body wt), sat for 1 hr in air (preimmersion), were immersed in water to the neck for 3 hr, and then sat for 1 hr in air (postimmersion). In another series they were similarly hydrated and then sat for 5 hr in air for the time control. Urine and venous blood samples were collected hourly for creatinine and electrolyte measurements. In addition, the concentration of ANF was determined in unextracted plasma by a radioimmunoassay. The pattern of electrolyte excretion was evaluated on the basis of fractional excretion of filtered load. In the time control series, urine flow and fractional excretion of Na and K remained low throughout the 5-hr experimental period. On the other hand, urine flow increased significantly from the preimmersion level of ~2 to ~7 ml/min during the first hour of immersion ($P < 0.05$), after which it decreased to ~5 ml/min during the second hour of immersion ($P < 0.05$) and to ~2 ml/min during the third hour of immersion. Fractional excretion of Na increased continuously from preimmersion level of ~1.0 to ~1.8% during the second and third hours of immersion ($P < 0.05$) and then decreased to 1.2% during the 1-hr postimmersion period. The plasma ANF remained low (~75 pg/ml) during the 5-hr time control period. In the immersion series, plasma ANF increased significantly from the preimmersion level of ~80 to ~120 pg/ml during the entire 3-hr immersion period and then returned to the preimmersion level during 1 hr postimmersion. These results indicate that the immersion diuresis and natriuresis are indeed associated with the increased ANF release. However, it can not be ascertained from the present study if the increased ANF contributes directly to these renal responses to immersion or in concert with other mediators. © 1987 Society for Experimental Biology and Medicine.

Head-out water immersion in thermoneutral water (~35°C) induces a significant increase in cardiac output, a diuresis and natriuresis (1). Although it is generally recognized that immersion-induced central hypervolemia is responsible for the above cardiac and renal responses, the exact mechanism for the renal responses are still under debate among investigators. Gauer and Henry (2) proposed that the cardiac and thoracic volume receptors are stimulated by the immersion-induced central hypervolemia, resulting in a reflex suppression of antidiuretic hormone (ADH) and an attendant diuresis. Indeed, the inhibition of the ADH system during immersion has been demonstrated as indicated by decreases in plasma ADH and/or urinary excretion of ADH (3-11). However, the HOI diuresis has also been shown to develop in the absence of any change in the ADH release (12, 13). Although the renin-aldosterone system is also suppressed during immersion (1, 5, 9, 14), the immersion natriuresis still persists, even when

the subject is pretreated with deoxycorticosterone acetate (DOCA) (14). Moreover, Epstein *et al.* (15) found a natriuretic factor in the urine of subjects during immersion; however, the exact nature of this natriuretic factor has not been determined.

In 1981, de Bold *et al.* (16) reported that an intravenous injection of atrial myocardial extracts induced a rapid and potent natriuretic response in rats, and the substance responsible for this action was termed "atrial natriuretic factor (ANF)." Since then, the presence of ANF has been documented in many other species, including humans (17-19). Although the exact mechanisms underlying the release and the natriuretic effect of ANF are not clearly understood, it appears that atrial stretch is associated with the release of ANF from atrial granules (20, 21). Since immersion is associated with atrial distension, it is reasonable to assume that ANF may be released during immersion and lead to the development of a natriuresis. Indeed, Epstein *et al.* (22) observed

a prompt and marked increase in plasma ANF in four human subjects undergoing a 3-hr immersion, which supported the hypothesis that an increase in plasma ANF contributes to the hormonal and renal effects of immersion in water to the neck. Although similar increases in plasma ANF were also observed by Miki *et al.* (23) in conscious dogs during immersion, the time course and magnitude of the changes in plasma ANF and natriuresis during immersion were not comparable. The present investigation was undertaken to investigate the functional relationships between ANF and the natriuresis and diuresis that occur during immersion.

Materials and Methods. Six men were used as subjects after description of the experiment, familiarization with procedures, and voluntary informed consent. They were healthy non-smokers who had participated in an earlier series of immersion experiments (5). On the average (\pm SE), they were 24.0 ± 0.8 years old, 181 ± 3 cm in height, and 96 ± 4 kg in weight, with a maximal aerobic power of 58.5 ± 4.0 ml O₂/kg/min. None of these subjects was actively engaged in sport competition or training at the time of the experiment.

Each subject participated in two protocols that were carried out in a random order. In one protocol (time control), they remained in the seated position in air for 5 hr; in the other protocol (immersion), the subjects were seated for 1 hr in air (preimmersion), immersed to the neck in the seated position in $35 \pm 0.05^\circ\text{C}$ water for the next 3 hr, and then returned to the seated position in air for the final hour (postimmersion). Both urine and venous blood (6 ml) were collected at the end of each hour. Room air was maintained at 24°C .

The subjects reported to the laboratory at 0800 hr after 10 hr of fasting and no physical activity. The subjects emptied their bladder, drank a bolus of water (equivalent to 0.5% body wt) in order to produce a uniformly hydrated state, and then were seated for the first hour. No additional fluid or food was ingested during the experiment.

For the measurement of ANF, 4 ml of blood sample was placed in separate prechilled tubes containing sodium ethylenediaminetetraacetic acid (Na-EDTA, 10.5 mg). The samples were centrifuged at 4°C , and the plasma was removed and stored at -70°C until it was an-

alyzed by radioimmunoassay (see below). The remaining 2 ml of blood was collected in a chilled tube containing lithium heparin, centrifuged, the plasma was removed and the sample was analyzed. Plasma and urine were analyzed for sodium and potassium concentrations (Radiometer, flame photometer, Model FLM 3) and osmolality (Precision system, freezing point osmometer, Model 5004). Colorimetric determination of creatinine concentration was by the Jaffe reaction (24).

ANF radioimmunoassays were carried out according to a method using unextracted plasma samples which is described in detail elsewhere (23). The antisera, obtained from rabbits immunized with a cardionatrin I (CI), cross-reacted 100% with α -human ANF and showed no detectable cross-reactivity with other commercially available polypeptide hormones. Recovery of added CI from untreated plasma samples was $100.5 \pm 8.6\%$, and the sensitivity of the method was 1.5 pg/tube at 95% inhibition. The coefficient of variation was 3.8% (within assays) and 9.1% (between assays).

Statistical analysis was performed by two-way analysis of variance for repeated measures for the same subject (25). The two independent variables were the time course and experimental condition. Subsequent post hoc analysis to determine differences among the means was carried out by using Dunnett's test (26). The null hypothesis was rejected when $P < 0.05$. Values are reported as means \pm SE.

Results. Urine flow increased significantly during the first 2 hr of immersion as shown in Fig. 1a. However, urine flow returned to the preimmersion level during the third hour of immersion. On the other hand, urine flow remained low throughout the 5-hr time control period. Both pre- and postimmersion urine flows were not different between control and immersion series. At the peak of the immersion-induced diuresis, urine osmolality decreased significantly to 241 mOsm/kg from the preimmersion level of 636 mOsm/kg (Table I).

Creatinine clearance (C_{cr}) was higher during the first 2 hr of the experimental period in both protocols (Table I), most likely due to incomplete voiding of the bladder by the subjects prior to the bolus ingestion of water. However, C_{cr} values stabilized at 130 ml/min

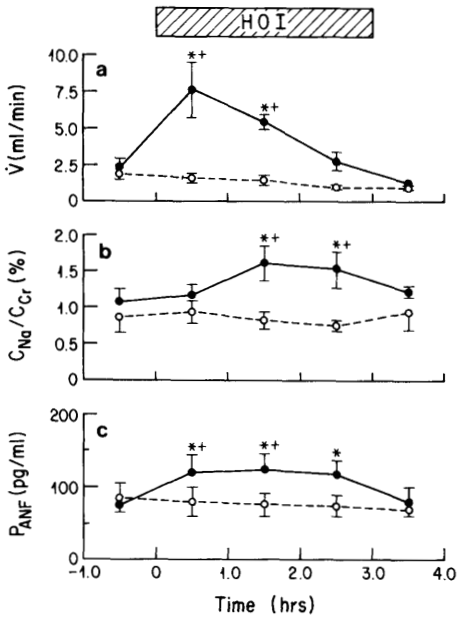


FIG. 1. Mean and SE for urine flow (a, \dot{V}), fractional sodium excretion (b, C_{Na}/C_{cr}), and plasma atrial natriuretic factor (c, P_{ANF}) plotted as a function of time. Head-out water immersion is represented by the solid lines and closed circles and the time control data by the dashed lines and open circles. Values that are significantly different* from the preimmersion values† from the time controls ($P < 0.05$).

after the third hour in both protocols. In order to eliminate the errors due to these time-dependent changes in C_{cr} , urinary excretion of osmotic particles and water is expressed as a fraction of the filtered load. However, the pattern of changes in the fractional excretion of water (\dot{V}/C_{cr}) during immersion was the same as that described above for urine flow (Fig. 1a and Table I). The fractional excretion of osmotic substances (C_{osm}/C_{cr}) during immersion was not significantly greater than during time control (Table I). However, the fractional excretion of Na (C_{Na}/C_{cr}) increased significantly by 50% during the second and third hours of immersion and then it decreased to the preimmersion level upon termination of immersion (Fig. 1b). The absolute rate of Na excretion during the second and third hours of immersion (327 ± 72 and $301 \pm 68 \mu\text{Eq}/\text{min}$, respectively) was also significantly greater ($P < 0.05$) as compared to the corresponding timed-control values (151 ± 27 and $136 \pm 18 \mu\text{Eq}/\text{min}$, respectively). Although the frac-

tional excretion of K (C_K/C_{cr}) and the free water excretion relative to C_{cr} (C_{H_2O}/C_{cr}) also increased substantially during the first 2 hr of immersion (Table I), these increases were not significant ($P > 0.05$).

The average plasma ANF concentration (P_{ANF}) was maintained at ~ 80 pg/ml during 5 hr of the time control. However, P_{ANF} was significantly increased to ~ 120 pg/ml at 1 hr immersion sample time, remained at this elevated level during the entire immersion period, and then returned to the preimmersion by the end of 1 hr postimmersion (Fig. 1c). The increased pattern of P_{ANF} during immersion was observed in all of the six subjects, although the magnitude of the increase (the 3-hr average for the immersion period minus the preimmersion value) varied from 3 to 88 pg/ml.

Both plasma osmolality and Na concentration were maintained constant throughout 5 hr at ~ 287 mOsm/kg and ~ 139 mEq/liter, respectively, in both protocols. Although plasma K concentration was maintained constant at ~ 4.4 mEq/liter in the time control series, it decreased slightly from 4.3 mEq/liter during preimmersion to 4.0 mEq/liter at the end of 5 hr in the immersion series ($P < 0.05$).

To determine if there were a significant relationship between changes in plasma ANF and Na excretion during immersion, the fractional excretion of Na for individual subjects from all experiments was evaluated as a function of P_{ANF} . As shown by the broken line in Fig. 2, there was a significant positive correlation between C_{Na}/C_{cr} and P_{ANF} ($r = 0.47$; $P < 0.001$). This significant correlation, however, had only a 22% shared variance. In fact, when the individual data obtained from the immersion series were plotted, four out of six subjects increased both P_{ANF} and C_{Na}/C_{cr} during immersion and two subjects (indicated by O's and X's) did not increase C_{Na}/C_{cr} despite the substantial increase in P_{ANF} (by 25–75 pg/ml) during immersion. In other words, while there may be an overall correlation between C_{Na}/C_{cr} and P_{ANF} , the relationship is not tight enough to be applied to individual cases.

Discussion. The present work was undertaken to define the role of ANF in the development of the immersion natriuresis. Previous studies conducted in our own laboratory have shown that plasma renin activity (PRA) as well

TABLE I. VARIOUS RENAL FUNCTIONS DURING HOI

Experimental series	Preimmersion, 1 hr	HOI			Postimmersion, 5 hr
		2 hr	3 hr	4 hr	
Uosm (mOsm/kg)					
Control	755 ± 135	733 ± 96	739 ± 120	790 ± 85	806 ± 94
HOI	636 ± 90	257 ± 112***	241 ± 19***	411 ± 17***	642 ± 43
Ccr (ml/min)					
Control	196 ± 42	170 ± 34	132 ± 8	130 ± 12	130 ± 21
HOI	192 ± 46	160 ± 14	139 ± 10**	138 ± 9**	118 ± 16***
\dot{V}/Ccr (%)					
Control	1.09 ± 0.31	1.08 ± 0.20	1.12 ± 0.27	0.86 ± 0.14	0.81 ± 0.15
HOI	1.44 ± 0.23	4.61 ± 0.97***	3.80 ± 0.27***	1.99 ± 0.23***	1.04 ± 0.10*
Cosm/Ccr (%)					
Control	2.36 ± 0.48	2.52 ± 0.43	2.36 ± 0.33	2.23 ± 0.26	2.51 ± 0.46
HOI	2.91 ± 0.27	2.64 ± 0.26	3.15 ± 0.27	2.82 ± 0.31	2.27 ± 0.08
CK/Ccr (%)					
Control	11.7 ± 1.6	15.0 ± 2.2	15.8 ± 2.0	16.3 ± 2.2	16.9 ± 1.8
HOI	14.0 ± 2.7	20.8 ± 3.0	25.3 ± 2.4	20.1 ± 2.2	14.4 ± 2.3
CH ₂ O/Cr (%)					
Control	-1.30 ± 0.50	-1.44 ± 0.33	-1.24 ± 0.31	-1.40 ± 0.26	-1.70 ± 0.31
HOI	-1.47 ± 0.18	1.97 ± 1.11	0.64 ± 0.25	-0.84 ± 0.13	-1.23 ± 0.06

Note. Data are means ± SE.

* Significantly different from corresponding time control ($P < 0.05$)

** Significantly different from corresponding 1-hr value ($P < 0.05$)

as urinary and/or plasma aldosterone level decreased significantly during immersion (5, 9), which is in agreement with the findings by

other investigators (1, 14). However, we also found that such inhibition of PRA and plasma aldosterone during immersion was still present

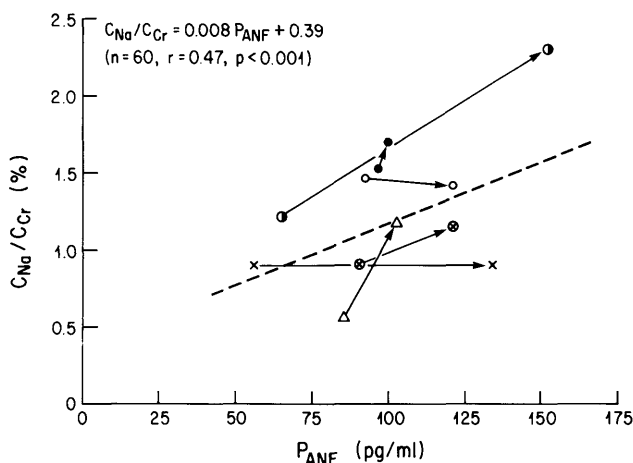


FIG. 2. Fractional sodium excretion (C_{Na}/C_{Cr}) is plotted as a function of plasma atrial natriuretic factor. The dashed line and regression results in upper left of figure is the line of best fit through the individual C_{Na}/C_{Cr} and P_{ANF} values for each subject at each hour in both protocols. All subjects are equally represented as there was no missing data. The solid lines are individual subjects data prior to (preimmersion) and during immersion (3-hr average).

even at night when the immersion natriuresis was markedly attenuated (9). In earlier studies, Epstein *et al.* (14) also reported that subjects pretreated with a pharmacological dose of DOCA still developed a marked natriuresis during immersion and concluded that aldosterone suppression alone cannot completely account for the immersion natriuresis. Subsequently, Epstein *et al.* (15, 27) suggested that increased activity of the natriuretic factor or renal prostaglandins may also constitute a determinant of the immersion natriuresis. More recently, Epstein *et al.* (22) reported that an increase in plasma ANF also contributes to the hormonal and renal effects of immersion in humans. Miki *et al.* (23) in our laboratory also observed an increase in plasma ANF in conscious dogs during immersion, although the time course and magnitude of the changes in plasma ANF and natriuresis during immersion were different.

The present study also showed significant increases of plasma ANF during immersion, confirming earlier findings (22, 23). In fact, in both the present and earlier studies, the immersion induced a prompt and sustained increase in plasma ANF during immersion, which was accompanied by reciprocal changes in plasma renin and aldosterone (22). However, the relative magnitude of the increase in plasma ANF induced by immersion was greater in the earlier study (100–300%) (22) as compared to the present study (50%). Although the reason for this difference between the two studies is not obvious, it may be at least in part accounted for by the difference in the degree of hydration of the subjects in the two studies. In the earlier immersion study in which ANF was measured (22), each subject drank 200 ml of water hourly (or 1000 ml during 5 hr of experimental period) while, in the present study, each subject was hydrated with a bolus of water equivalent to 0.5% body weight (450 ml on the average) only once at the beginning of the preimmersion period. The high urine flow was sustained during immersion in the earlier study (22) while the magnitude of diuresis decreased continuously during the course of immersion in the present study (Fig. 1). In an earlier study, we also noted that our subjects given only one bolus of water, as in the present study, failed to sustain the increased cardiac output during immersion

(5), in contrast to other studies in which continuously hydrated subjects sustained the increased cardiac output during 4 hr of immersion (28, 29). These observations suggest that the degree of central hypervolemia may be dependent upon the degree of hydration. In fact, in one series of studies, we also continuously hydrated the subjects to maintain a constant body fluid volume during immersion, and noted that both the diuresis and the increased cardiac output were sustained during 3 hr of immersion (unpublished data of D. R. Pendergast *et al.*).

The increase in plasma ANF observed during immersion is indeed consistent with the view that an increase in central blood volume (or atrial stretch) is one mechanism promoting ANF release in humans and also strongly suggests that ANF may play an important role in the development of the immersion natriuresis. It should be noted, however, that the time course of changes in plasma ANF during immersion is different from that of the natriuresis. The natriuresis was not significant during the first hour of immersion and it peaked and was significant during the second hour (Fig. 1b). As mentioned above, Miki *et al.* (23) reported that, although immersion increased both Na excretion and plasma ANF, the time course and magnitude of the changes in plasma ANF and Na excretion during immersion are not comparable in conscious dogs. A careful inspection of the data obtained from the six individuals employed in the present study revealed that, while immersion induced an increase in plasma ANF in all subjects, this increase was associated with an increase in fractional Na excretion in only four subjects; in the remaining two subjects, plasma ANF increased substantially during immersion without increasing the fractional Na excretion (Fig. 2). Recently, Goetz *et al.* (20) observed in conscious dogs that elevation of left atrial pressure consistently increased plasma ANF, urine flow and Na excretion, but surprisingly, elevation of right atrial pressure in the same dogs consistently increased plasma ANF but did not increase urine flow or Na excretion. These investigators (20) further noted that elevation of left atrial pressure in conscious cardiac-denervated dogs also resulted in increased plasma ANF but not urine flow or Na excretion. As described above, the immersion na-

triuresis is markedly attenuated at night as compared to that during the day (9, 30). Despite this difference in the magnitude of immersion natriuresis between day and night, the plasma ANF level increased during immersion in an identical manner during both day and night (unpublished data of K. Shiraki *et al.*). These results clearly indicate that ANF is indeed released into the circulation by atrial distension but that it is not necessarily responsible for the immersion natriuresis. It is conceivable that ANF released during atrial distension is likely to act in concert with other mediators such as renin-angiotensin-aldosterone, ADH, and renal prostaglandins in modulating the immersion natriuresis, as suggested by Epstein *et al.* (22).

Although ANF is considered to be a natriuretic factor, as the name implies, it may also play an indirect role in modulating the excretion of free water. Recently, Dillingham and Anderson (31) reported that ANF inhibits the hydrosmotic action of ADH in the rabbit collecting tubules perfused *in vitro*. A similar inhibition of the hydrosmotic action of ADH by ANF was also found in the isolated toad urinary bladder (32). These findings suggest a potential role for inhibition of the renal response to ADH by ANF as a contributor to a diuretic state. As discussed above, the immersion diuresis is often, but not always, associated with the reduction of plasma and/or urinary ADH (3-11). It is quite conceivable that the elevated level of plasma ANF in response to immersion interferes with the free water reabsorption in the distal nephron even when the plasma level of ADH is not suppressed. This should lead to an increase in free water clearance as found in the present and previous studies.

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