JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2012, 63, 1, 87-94
www.jpp.krakow.pl

S.Y. CHUNG[5+], S.K. KIM[4+], CH.W. HONG[3], K.W. OH[2], K.T. KIM[2], J.G. SUL[2], J.W. CHUNG[2], M.S. KWON[1]

THE TIME-DEPENDENT ALTERATION OF ANTI-DIURETIC HORMONE SYSTEM IN HINDLIMB UNLOADED RATS

[1]Department of Pharmacology, School of Medicine, CHA University, Bundang-gu, Seongnam, Gyeonggi-do, South Korea;
[2]Aerospace Medical Research Center, ROKAF, Cheongwon-gun, Chungcheongbuk-Do, South Korea;
[3]Department of Chemical and Biological Warfare Research, Armed Forces, Medical Research Institute, Daejeon, South Korea;
[4]Department of Internal Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Ji-Dong, Paldal-gu, Suwon, Gyeonggi do, South Korea; [5]Department of Pathology, Dongnam Institute of Radiological & Medical Sciences, Jangan-eup, Gijang-gun, Busan, South Korea

It is important to understand the mechanism on the fluid shift and volume regulation occurring in astronauts after spaceflight for future life in space. In the present study, we examined the time-dependent alteration of anti-diuretic hormone (ADH) concentrating on the water reabsorption system in hindlimb unloaded rats. Male Sprague-Dawley rats were hindlimb unloaded for 1 (HU1), 7(HU7), 14 days (HU14) or rested in the ground for 3 days after HU14 (HU14+3). The plasma ADH and angiotensin II level showed peak value at HU7, and the alterations were restored at HU14. However, several serum electrolytes (Na, K, Cl) were not changed regardless of HU period. In the immunohistochemical study, we examined that ADH and c-Fos immunoreactivities (IR) were maximized at HU7 in the paraventricular nucleus (PVN) and supraoptic nucleus (SON). Aquaporin 2 (AQP2) IR also was increased in the renal collecting duct for water re-absorption at HU7 showing a similar pattern with ADH. These results present a series of physiological ADH system alteration following to period of hindlimb unloading stimulus, indicating that ADH system is activated significantly at HU7. In addition, our results suggest that ADH system activation may be involved in anti-diuretic phenomenon in early spaceflight period. Furthermore, it is speculated that ADH system may require 14 days for adaptation to microgravity.

Key words: *angiotensin II, anti-diuretic hormone, aquaporin-2, hindlimb unloading model, hypothalamus, blood urea nitrogen*

INTRODUCTION

The hindlimb unloaded (HU) rat model has been accepted by the scientific community as the rodent model of choice for simulating physiological effects of weightlessness/microgravity, and its use will likely increase during the space station era. A variety of studies have demonstrated an alteration of several physiological properties in the HU rat. Especially, the HU rat model has been used to examine several changes associated with spaceflight, including fluid and electrolyte homeostasis (1, 2). Recently, a series of studies have reported that there are evidences that these physiological alterations produced by hindlimb unloading may be due to centrally mediated alterations partly (3, 4).

Maintaining water homeostasis as controlling both osmolality and intravascular blood volume is essential for terrestrial mammals to survive. During usual activity, water homeostasis processes are tightly controlled by regulating both water intake and urinary water excretion. Especially, vascular volume and baroreceptors regulating the release of the antidiuretic hormone (ADH) sense the changes in intravascular blood volume (5). Noticeable alterations in the regulation of fluid and electrolyte homeostasis have also been observed in humans during spaceflight (6).

ADH, also known as vasopressin, is synthesized in the hypothalamus, and secreted from the posterior pituitary to blood stream. It has been well known that change of osmolality, plasma volume, or redistribution of blood (7, 8) leads to ADH secretion by osmoreceptors located within specific regions of the hypothalamus and by pressure receptors in the veins, atria, and carotids, or intrathoracic stretch receptor. ADH binds its receptor (vasopressin V2 receptor) in the collecting duct, initiating a signal transduction cascade. Consequently, aquaporin-2 (AQP2) water channels are phosphorylated and translocated to apical plasma membrane to increase water permeability (9). Thus, water can be reabsorbed in the renal collecting duct by ADH-dependence on the body's need.

At the onset of spaceflight, there is a cephalic shift of fluids in humans. It has been well known in human subjects that the body fluid balance is distributed for a few days after an exposure to microgravity. However, the reduced urine volume is observed in the spaceflight against a general expectation (diuresis induced by a cephalic shift of fluids) following the Henry-Gauer reflex (10). To confirm the role of ADH in this paradoxical phenomenon, we aimed to evaluate the renal responses in rats following spaceflight in HU rat model, especially focusing to

+ S.Y. Chung and S.K. Kim are both co-first autor.

time-dependent alteration of ADH system from brain to kidney. In the present study, our results suggest that ADH system activation may be involved in anti-diuretic phenomenon in early spaceflight period. In addition, it is speculated that ADH system may require 14 days for adaptation to microgravity.

MATERIALS AND METHODS

Experimental procedures

These experiments were approved by the Aerospace medical research center "Animal Care and Use Committee (South Korea)". All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Experimental animals

Male Sprague-Dawley (SD) rats (Samtako Co., Daejeon, Korea) weighing 250-260 g were used for all the experiments. After adaptation for 6 days, animals were housed one per cage or HU cage in a room maintained at 22±0.5°C with an alternating 12 h light-dark cycle. Food and water were available ad libitum. Animals were allowed to acclimate to the laboratory 6 days before the beginning of the experiments. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were only used once. To reduce variation, all experiments except hindlimb loading were performed during the light phase of the cycle (10:00-12:00). The animal number was 9-12 per group.

Hindlimb unloading model

Male Sprague-Dawley rats were hindlimb unloaded for 1 (HU1), 7(HU7), 14 days (HU14) or rested in the ground for 3 days after HU14 (HU14+3) according to previously published methods (11). In brief, animals underwent an acclimation period during which they were suspended by the tail for short durations (1-3 h/day). Following 3 days of acclimation, animals were tail suspended on the fourth day by either tail hardness or a tail stainless steel rings. Tail harnesses or rings were attached under halothane anesthesia (2%), and procedures took less than 10 min Animals were subjected to tail suspension to partially elevate the hind limbs above the floor of the cage. Generally, this system involves a cage with rigid parallel walls and a cross bar with a wheel assembly at each end riding on the top edges of opposite walls of the cage. Briefly, the tail was cleaned and dried. Adhesive sponge tape strips (Weather strips, Daesung Co., Chungnam, Republic of Korea) the width of the tail were adhered laterally along the two sides of the proximal two-thirds of the tail. Theses longitudinal strips were then secured to the tail by three 1 cm wide tape strips (Cole-Parmer International, Vernon Hills, IL) wrapped circumferentially at three sites along the length of the tail. The rats were suspended *via* a small chain was rolled freely along the length of the crossbar at the top of the cage. The floor of the cage was made of parallel plastic bars, 1 cm in diameter, with spaces between them. These designs allowed the rats free range of movement around the cage and prevented them from grasping the cage floor and pulling to decrease the traction force on the tail. Adjustments to the length of the chain were made as necessary to prevent the rats' hindlimbs from touching any supportive surfaces while the forelimbs maintained contact with the cage floor. The animals were maintained in a ~30 head-down tilt (*i.e.*, the angle formed between the torso of the animal and the floor of the cage), with the hind limbs elevated ~0.5 cm above the floor when fully extended. The control animals were maintained in the same environment with HU rat, except hindlimb unloading. The animals were randomly grouped, and the cardiac puncture and perfusion were conducted in all groups at same day. After HU period, the rats were anesthetized with pentobarbital (50 mg/kg) intraperitoneally, and then prepared to blood sample and perfusion. Animals were monitored on a daily basis. Normal food and water intake, grooming, defecation, and urination were used as indications that animals were not under overt stress. On the basis of a previous study using this criteria, there was no difference in adrenal gland weight between control and HU rats (12), which suggests animals used in the current study were not under overt chronic stress. There were no significant difference in body weight and water intake between control and HU rats at time point we observed for 14 days (13). We consider that the stress of the i.p. injection, duration of time to sedation, and effect of cardiac puncture on ADH and angiotensin II level can be all variables. Thus, we tried to minimize i.p. stress of rats and to maintain consistent cardiac puncture technique and duration of time to sedation (5 min per rat for anesthesia) in all groups.

Anti-diuretic hormone and angiotensin II determination

Blood was collected by cardiac puncture (left ventricle) with 10 mL syringe, and plasma was separated by centrifuge. Collected plasma was used for ADH or angiotensin II determination. ADH was measured by vasopressin direct RIA kit (Buhlmann, Swiss). Angiotensin II was measured by ELISA kit (phoenix pharmaceuticals, USA). All methodologies are well described in the manufacturer instructions. Cobra r-counter, USA

Blood biochemistry

Blood was collected by cardiac puncture (left ventricle) with 10 mL syringe and serum was separated from cells. Blood biochemistry determinations were performed manually with a chemistry analyzer (Olympus AU400, Olympus Co., Japan). Parameters were sodium, potassium, chloride, blood urea nitrogen, creatinine. All methodologies are well described in the manufacturer instructions.

Immunohistochemistry and c-Fos or anti-diuretic hormone positive cell counting in paraventricular and supraoptic nucleus

For perfusion, all rats were first deeply anesthetized (duration: 5 min) with sodium pentobarbital (50 mg/kg), *i.p.*, and perfused intracardially with physiological saline followed with ice-cold phosphate-buffered 4% paraformaldehyde (pH 7.4). Whole brain and kidney was dissected and post-fixed in the same fixative for 4 h at 4°C. Then the brain blocks were cryoprotected in 30% sucrose for 24 h at 4°C. The fixed tissues were embedded in paraffin and 3.5 μm (kidney) and 15 μm (brain) thick tissue sections were stained. Immuno-histochemical staining was performed with the Zymed nonbiotin amplification system (Zymed Laboratories Inc, South San Francisco, CA). Briefly, tissue sections were dewaxed in xylen for more than 20 minutes and sequentially hydrated in 100%, 95%, 90%, and 80% ethanol solution. After rinsing with water for 5 minutes, the sections were then pretreated with 0.01 mol/L sodium citrate buffer and autoclaved for 1 minute to retrieve the antigen. After rinsing, the endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 30 minutes. The polyclonal anti-rabbit c-Fos (1:1000; Santa

Cruz, USA), anti-rabbit vasopressin (1:1000, Abcam,Cambridge,UK) or aquaporin II (1:4000, Abcam,Cambridge,UK) were applied to the sections overnight in a moist chamber at 4°C. After rinsing with phosphate-buffered saline, the slides were incubated with secondary antibody for 10 minutes at room temperature and rinsed with phosphate-buffered saline. Sections were incubated in tertiary antibody-horse radish peroxidase conjugate for 10 minutes, rinsed in phosphate-buffered saline, and incubated with diaminobenzidine for 10 minutes. After counterstaining with Meyer's hematoxylin, the sections were dehydrated through graded ethanols, cleared in histoclear (Fisher, USA), and coverslipped using Permount (Fisher, USA).

Histological analysis method in brain regions we observed was performed following under procedures. The number of c-Fos or ADH immunoreactive nuclei was counted by two blinded observers at the same time using an image analyzing system equipped with a computer-based CCD camera (Olympus AX70, USA). The number of c-Fos or ADH immunoreactive nuclei was counted in three sections in reference of the rat brain atlas (14) for each animal. Starting from the first section (PVN and SON : interaural 7.60 mm, Bregma -1.40 mm), counts were taken from at least three coronal sections at 45 μm increments. Thus, we could always perform cell counting of the same brain region and minimize any counting bias. The number of c-Fos or ADH IR positive cells was compared to that of the control group of the same brain area from all animals. The number of animals used for each group was 9-12.

Statistical analysis

Data were presented as the mean ±S.E.M. The statistical significance of differences between groups was assessed with one-way ANOVA with a Bonferroni post hoc test using GraphPad Prism version 4.0 for Windows XP (GraphPad Software, San Diego, CA, USA); $P<0.05$ was considered significant.

RESULTS

The time-dependent alteration of body weight, plasma ADH, serum sodium, potassium, chlolide, blood urea nitrogen (BUN), and creatinine (Cr) in HU rats

It has been demonstrated that tail traction method we used for hindlimb unloading appears to be less stressful to animals, as assessed by corticosterone levels and adrenal, thymus, and body weights (15, 16). In according with these studies, the difference of body weight between control rats and hindlimb unloaded rats was not significant (*Fig. 1B*). To clarify the role of ADH in paradoxical phenomenon against the Henry-Gauer reflex during the spaceflight (10), we first examined the time-dependent alteration of plasma ADH level in HU rats. The plasma was collected from each group (control, HU1, HU7, HU14, HU14+3) at the same day, and analyzed by RIA kit. Because the stress of the i.p. injection, duration of time to sedation, and effect of cardiac puncture on ADH are all variables to consider, We tried to minimize i.p. stress of rats and maintain consistent cardiac puncture technique and duration of time to sedation (5 min per rat) in all groups. The absolute plasma ADH level was not different from a previous study (17). As shown in *Fig. 1C*, the plasma ADH level was elevated significantly at HU7 and hormone level of other groups was similar to control group. To ascertain factors (especially, osmolality and prerenal blood flow) associated with plasma ADH alteration in HU rat, we observed the alteration of serum electrolytes (Na, K, Cl), BUN, and Cr. As shown in *Fig. 2*, serum Na, K, and Cl concentration were not changed regardless of HU period. However, serum BUN was elevated at HU7 and restored at HU14 without serum Cr alteration. Thus, the serum BUN/Cr ratio was also peak at HU7.

The time-dependent alteration of plasma angiotensin II in hindlimb unloaded rats

It has been demonstrated that angiotensin II (ANG II) interacts with ADH on water reabsorption in collecting duct (18).

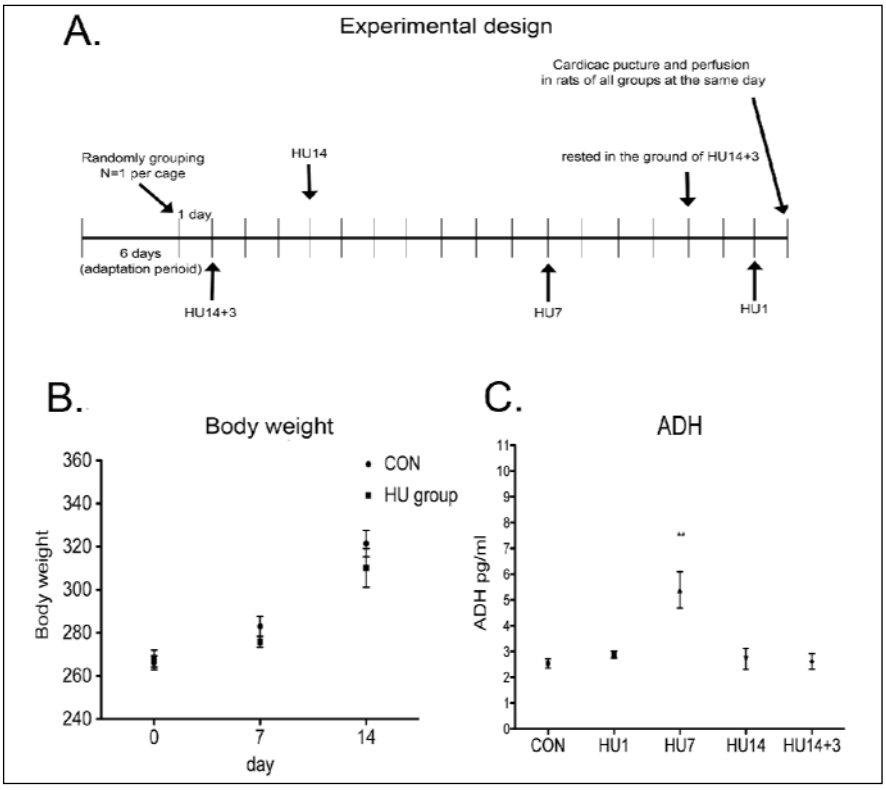

Fig. 1. The experimental design (A), the alteration of body weight (B) and plasma anti-diuretic hormone (ADH) level (C) following to hindlimb unloading period. Plasma ADH was determined by vasopressin direct RIA kit. CON (control group), HU1 (group hindlimbed for 1 day), HU7 (group hindlimbed for 7 days), HU14 (group hindlimbed for 14 days), HU14+3 (group rested in the groud for 3 days after HU14). *P<0.05 (CON *vs*. HU7), n=9 per group.

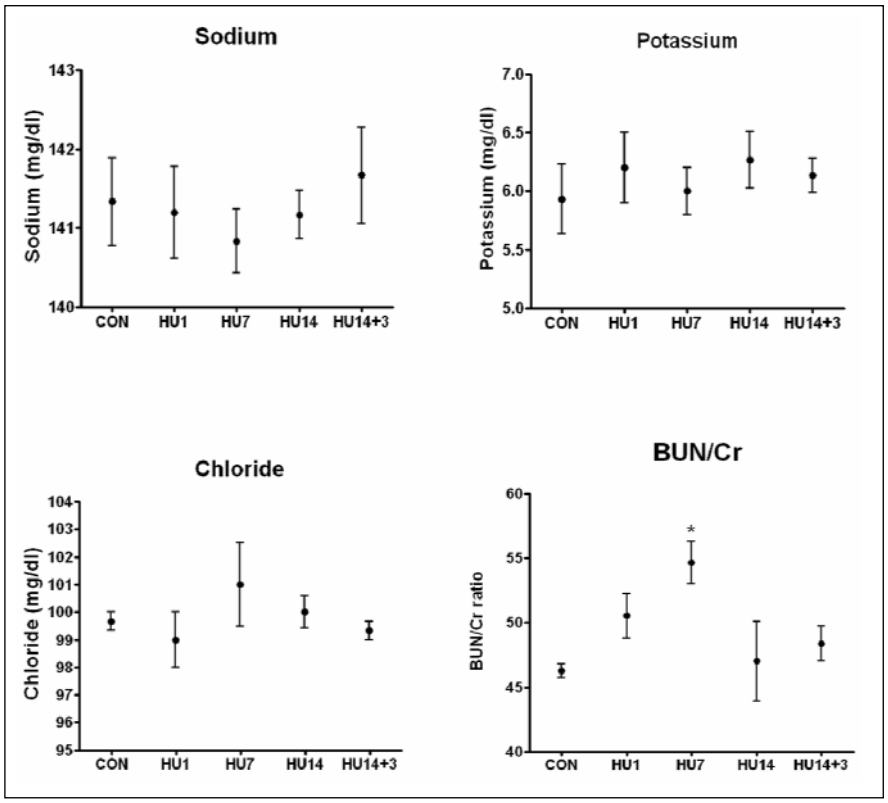

Fig. 2. The alteration of serum electrolytes (sodium, potassium, chloride) level and blood urea nitrogen (BUN)/creatinine (Cr) ratio following to hindlimb unloading period. Serum sodium (A), potassium (B), chloride (C) level, and BUN/Cr ratio was determined by a chemistry analyzer. CON (control group), HU1 (group hindlimbed for 1 day), HU7 (group hindlimbed for 7 days), HU14 (group hindlimbed for 14 days), HU14+3 (group rested in the groud for 3 days after HU14). *P<0.05 (CON *vs*. HU7), n=12 per group.

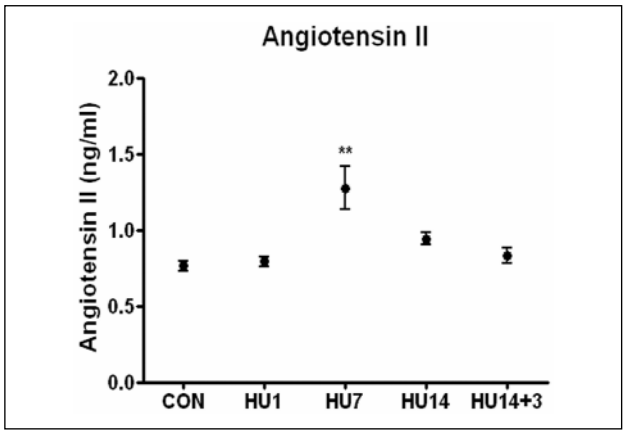

Fig. 3. The alteration of plasma angiotensin II (ANG II) level following to hindlimb unloading period. Plasma ANG II was determined by vasopressin direct RIA kit. CON (control group), HU1 (group hindlimbed for 1 day), HU7 (group hindlimbed for 7 days), HU14 (group hindlimbed for 14 days), HU14+3 (group rested in the groud for 3 days after HU14). **P<0.01 (CON *vs*. HU7), n=9 per group.

To observe the correlation between ADH and ANG II in HU rat we examined the time-dependent alteration of plasma ANG II in HU rats although we can not confirm whether ANG II plays a direct or indirect role on ADH alteration during HU. As shown in *Fig. 3*, plasma ANG II level showed a similar pattern with ADH indicating a significant increase at HU7 (*Fig. 1*).

The time-dependent alteration of anti-diuretic hormone and c-Fos immunoreactivities in the paraventricular and supraoptic nucleus

ADH is synthesized in PVN and SON of the hypothalamus, and secreted from the posterior pituitary to blood stream. The increased ADH production in the hypothalamus leads to plasma ADH level elevation. To confirm the relationship between plasma ADH elevation and ADH IR increase in the PVN and SON, we investigated an alteration of ADH IR in the PVN and SON of the hypothalamus following to HU period. As shown in *Fig. 4*, ADH IR in the PVN and SON showed a peak level at HU7 in agreement with *Fig. 1C*. We also examined the time dependent alteration of c-Fos IR in hindlimb unloaded rats, referring that c-Fos regulates ADH expression in PVN and SON (19). As shown in *Fig. 5*, c-Fos IR in the PVN and SON was increased significantly at HU7, showing a similar pattern with ADH IR alteration.

The time-dependent alteration of aquaporin 2 in collecting duct

Water is reabsorbed by AQP2 translocated to apical plasma membrane of the collecting duct. It has been reported that AQP2 is expressed in the plasma membrane by ADH. ADH, which is synthesized in PVN and SON of the hypothalamus, increases plasma ADH level and finally, induces an AQP2 expression in collecting duct of the kidney. Thus, we examined an alteration of AQP2 expression in collecting duct of the kidney to confirm whether a series of ADH elevation was involved in AQP2 expression at HU7. As shown in *Fig. 6*, AQP2 was increased significantly at HU7.

DISCUSSION

The cephalic fluid shift either induced by acute posture changes from upright to a supine body position (20), head-out water immersion (21), or acute isotonic saline infusion (22) leads to a natriuretic and diuretic response by the kidney, following the Henry-Gauer reflex (10). The central hypervolemia on Earth increases central venous pressure, provokes stretching of heart muscle, and stimulates a neurogen and/or hormone secretion to the kidney. However, a series of observations from space

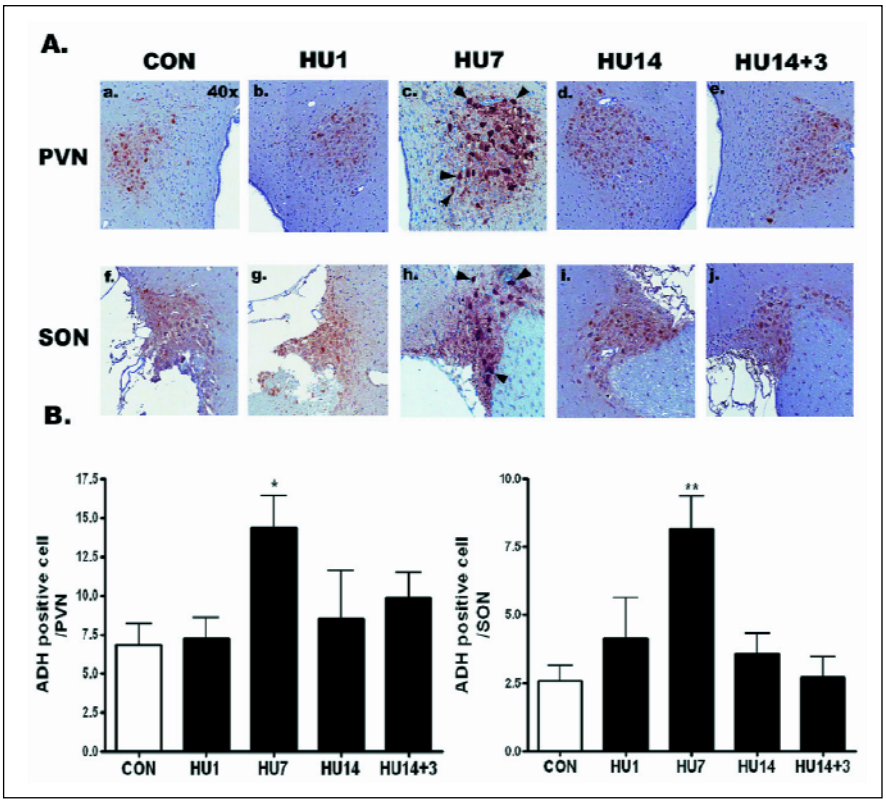

Fig. 4. The alteration of anti-diuretic hormone (ADH) positive cell number in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) following to hindlimb unloading period. An immunohistochemical study for ADH was performed in the PVN and SON. (A) ADH positive cell number (PVN and SON) is a little detected in the control (a, f) and HU1 (b, g) group. Significant ADH positive cell number elevation (PVN and SON) is detected in the HU7 (c, h) group. Both HU14 (d, i) and HU14+3 (e, j) groups (PVN and SON) shows a little ADH positive cell number. (B) The ADH positive cells in PVN and SON were counted referencing to the rat brain atlas (14). The vertical bars in the column graph indicate the standard error of the means. *P<0.05, **P<0.01 (CON *vs*. HU7). n=9 per group.

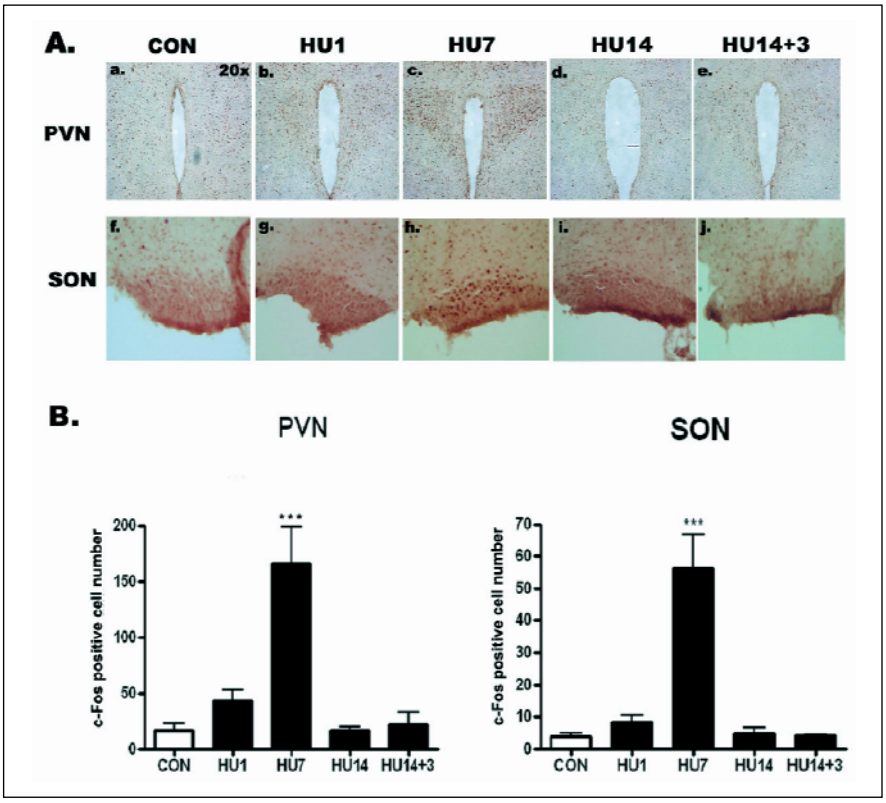

Fig. 5. The alteration of c-Fos positive cell number in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) following to hindlimb unloading period. An immunohistochemical study for c-Fos was performed in the PVN and SON. (A) c-Fos positive cell number (PVN and SON) is a little detected in the control (a, f) and HU1 (b, g) group. Significant c-Fos positive cell number elevation (PVN and SON) is detected in the HU7 (c, h) group. Both HU14 (d, i) and HU14+3 (e, j) groups (PVN and SON) shows a little c-Fos positive cell number. (B) The c-Fos positive cells in PVN and SON were counted referencing to the rat brain atlas (15). The vertical bars in the column graph indicate the standard error of the means. ***P<0.001 (CON *vs*. HU7), n=9 per group.

missions indicated that an exaggerated diuresis and natriuresis was never observed at the begging of the space flights (6, 23-25). In the present study, we observed that plasma ADH level was elevated at HU7, and the elevation was restored at HU14 indicating that ADH level elevation may be related to absence of diuresis and natriuresis at space flight. These results have some differences from previous report that HU14 shows higher ADH level compared to control group (26). Although we can not confirm exactly why this discrepancy occurs, it may be due to differences of hindlimb unloading method, animal age, weight, and whether animals have surgical procedures during HU. It has been well known that ADH is secreted by change of osmolality, total body water, redistribution of plasma volume. Among them, electrolytes (Na, K, Cl) associated with serum osmolality was not

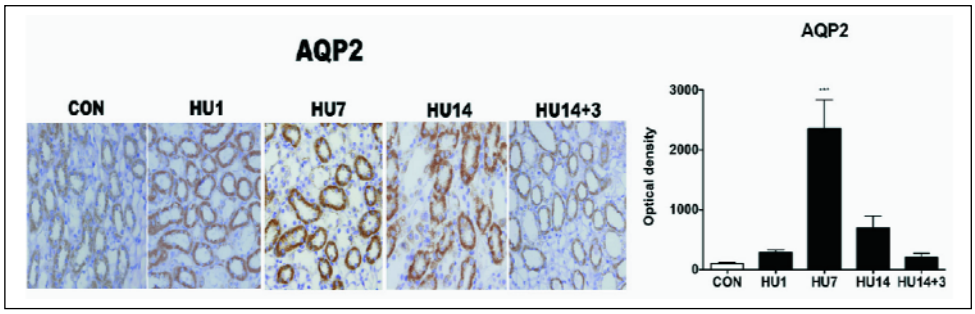

Fig. 6. The alteration of aquaporin 2 (AQP 2) immunoreactivities (IR) in the collecting duct of kidney. An immunohistochemical study for AQP II was performed in the collecting duct of kidney. Significant AQP 2 IR elevation was detected in HU7, n=9 per group.

changed by HU in the present study. This result is line with previous animal or human studies. Although it was not a time-dependent study, Wade *et al*. (27) observed that serum osmolality and serum concentrations of creatinine, sodium, potassium, and calcium at HU14 were not significantly different from those of control group. In addition, Leach *et al*. (25) observed that serum osmolality remained unchanged in an experiment with seven subjects during the Spacelab Life Sciences-1 (9 days) and -2 (14 days) missions. Taken together, these results support that serum osmolality may not be involved in the alteration of plasma ADH in HU rat although the direct osmolality will be checked in the further study.

It has been well reported that a 10-15% total body water reduction was observed in Spacelab Life Sciences (SLS) astronaut subjects (25). This decrease was evoked 21 h after launch, remaining below preflight levels until after landing. However, there are some reports that fluid loss may not occur in spaceflight in quadrupeds such as the rat (27) although there is one report that observes the decrease of plasma volume in HU rats (28). In addition, we examined that serum BUN/Cr showed similar pattern with ADH alteration in HU rat. It is well known that the increase of serum BUN/Cr without creatinine alteration is evoked by decrease of prerenal blood flow, which is triggered by the "decreased" state of effective circulatory volume induced by blood redistribution (29). This blood redistribution may be facilitated by fluid transfer from the intravascular to interstitial compartment in the space (25), in contrast to the expectation that diuresis by Henry-Gauer reflex (10) may contribute to a decrease of plasma volume in space. In addition, several astronaut studies suggest that stress reactions may increase urinary ADH level, especially at early flight (24 h) when acceleration exposures and other stresses of launch and re-entry probably were evoked (30). However, stress itself appears not to affect to plasma ADH level in the present study because the time point showing the ADH elevation (HU7) was not an early time and it has been reported that HU model is not a stress model (6), although we cannot exclude the effect of oxidative stress in the brain of HU rats (31). In the present study, we also examined that an alteration of serum BUN showed a similar pattern with that of plasma ADH following to HU period. Therefore, our results suggest that fluid transfer from the intravascular to interstitial compartment triggers to the decrease of effective circulatory plasma volume, which induces a decrease of prerenal blood flow to kidney, finally increases serum BUN without creatinine change during HU. Taken together, our results indicates that an alteration of plasma ADH level may be controlled by fluid transfer from the intravascular to interstitial compartment, not by osmolarlity change or dehydration at HU rats.

In regard to a crosstalk between ADH and angiotensin II (ANG II), there are several evidences indicating their potential relationships on the AQP2 water channels in the kidney. ANG II has been shown to stimulate the vasopressin V2 receptor messenger RNA in the inner medullary collecting duct (32). The ANG II receptor (AT1) blocker losartan decreases ADH-mediated cAMP accumulation in the thick ascending limbs and normalizes the increased Na-K-2Cl cotransporter (NKCC2) in rats (18). Recently, it has been reported that ANG II can be involved in maximal urinary concentration and decrease in AQP2 protein expression and AQP2 trafficking to the apical membrane of the collecting duct principal cells, in rat model without significant changes of renal blood flow, serum creatinine, or creatinine clearance (33). It suggests that ANG II itself may contribute to ADH function in HU rat although we can not exclude the ANG II-independent ADH activation (34). We also examined that plasma ANG II had a similar pattern with ADH during HU. Although we can not confirm that ANG II plays a direct role on ADH alteration during HU, it is speculated that a reciprocal action between ADH and ANG II during HU may affect to an AQP2 expression and water reabsorption in the collecting duct. Recently, it has been reported that GnRH may be involved in stimulation of ADH secretion (35). Thus, further study will be needed about the role of a potentiated factor like GnRH on ADH secretion in HU rats.

In the present study, we examined that all parameters (ADH, BUN, ANG II, AQP2 in the collecting duct, or ADH IR in PVN and SON) showing a peak level at HU7 were restored at HU14 although the more exact quantifying method on ADH IR in the brain will be needed for the future study. Because the elevated plasma ADH appears to be induced by decreased circulatory plasma volume in the present study, the restored ADH level at HU14 may indicate the return to normal of redistributed plasma volume. However, we examined that the normalization was shown even at HU14 rats, not HU14+3. Thus, voluntary normalization of redistributed plasma volume does not seem to induce recover of increased plasma ADH level in HU rats. Next, the possibility of an ADH system adaptation in HU rats can be considered. Several reports have demonstrated that a decrease in the ability to handle a water load was examined in postspaceflight rats (36-38). Changes in the sensitivity of volume receptors or the sequestration of fluids in the periphery also may be examined in rats after spaceflight (39) in contrast to human. Taken together, it is speculated that the adaptation of HU rats on ADH system may bring out the normalization of elevated values (BUN, ANG II, ADH, AQP2). We examined that c-Fos IR (neuronal activity marker) (40) in PVN and SON also showed peak level at HU7, and was restored at HU14. In addition, adaptive changes in c-Fos expression following repeated stimuli have been characterized well in animal model. Furthermore, it has been reported that c-Fos regulates ADH expression in PVN and SON (19). Thus, it is speculated that the adaptation may be one candidate that is associated with a normalization of the activated ADH system at HU14 although we cannot exclude the possibilities that c-Fos expression may be involved in oxidative stress or oxytocin release in PVN.

Although we observed the ADH IR increase in PVN and SON at HU7, several considerations will be needed on ADH IR

increase in PVN and SON. There are neurons of three major types in PVN and SON, which are ADH-producing cells, oxytocin-producing cells, and both-producing cells. First, it is speculated that the increase of ADH IR indicates more ADH protein production because it is considered that all ADH neurons do not have ADH IR against ADH antibody in the normal physiological state. Second, the changing of cell phenotype may contributed to ADH IR increase. In this case, one of two hormones may be dominant in both (oxytocin, ADH)-producing cells of the PVN and SON at HU7. Third, there is a possibility that another type of neuron (oxytocin neuron) changes its phenotype to become an ADH expressing neuron. In the present study, we can not confirm which mechanism is associated with the increase of ADH IR in the PVN and SON. To distinguish these possibilities, the further study will be needed.

In conclusion, our results suggest that ADH system activation by decreased circulatory plasma volume (fluid transfer from the intravascular to interstitial compartment) may explain anti-diuretic phenomenon in early spaceflight period against the Henry-Gauer reflex (10). In addition, it is speculated that ADH system may require 14 days for adaptation to microgravity. However, the further study will be conducted to elucidate the exact mechanism of ADH alteration following to HU period.

Acknowledgement: This research was supported by Aerospace medical research grants in 2009 from Republic of Korea Air Force (ROKAF).

Conflict of interests: None declared.

REFERENCES

1. Convertino VA, Doerr DF, Eckberg DL, Fritsch JM, Vernikos-Danellis J. Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension. *J Appl Physiol* 1990; 68: 1458-1464.
2. Meck JV, Waters WW, Ziegler MG, *et al*. Mechanisms of postspaceflight orthostatic hypotension: low alpha1-adrenergic receptor responses before flight and central autonomic dysregulation postflight. *Am J Physiol Heart Circ Physiol* 2004; 286: H1486-H1495.
3. Deavers DR, Musacchia XJ, Meininger GA. Model for antiorthostatic hypokinesia: head-down tilt effects on water and salt excretion. *J Appl Physiol* 1980; 49: 576-582.
4. Tucker BJ, Mundy CA, Ziegler MG, Baylis C, Blantz RC. Head-down tilt and restraint on renal function and glomerular dynamics in the rat. *J Appl Physiol* 1987; 63: 505-513.
5. McKinley MJ, Cairns MJ, DA Denton DA, *et al*. Physiological and pathophysiological influences on thirst. *Physiol Behav* 2004; 81: 795-803.
6. Drummer C, Norsk P, Heer M. Water and sodium balance in space. *Am J Kidney Dis* 2001; 38: 684-690.
7. Baylis PH. Regulation of vasopressin secretion. *Baillieres Clin Endocrinol Metab* 1989; 3: 313-330.
8. Segar WE, Moore WW. The regulation of antidiuretic hormone release in man: I. Effects of change in position and ambient temperature on blood ADH levels. *J Clin Invest* 1968; 47: 2143-2151.
9. Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci USA* 1995; 92: 1013-1017.
10. Gauer OH, Henry JP. Neurohormonal control of plasma volume. *Int Rev Physiol* 1976; 9: 145-190.
11. Jung CK, Chung S, Lee YY, Hwang SH, Kang CS, Lee KY. Monocyte adhesion to endothelial cells increases with hind-limb unloading in rats. *Aviat Space Environ Med* 2005; 76: 720-725.
12. Foley CM, Mueller PJ, Hasser EM, Heesch CM. Hindlimb unloading and female gender attenuate baroreflex-mediated sympathoexcitation. *Am J Physiol Regul Integr Comp Physiol* 2005; 289: R1440-R1447.
13. Zhang LF, Cheng JH, Liu X, *et al*. Cardiovascular changes of conscious rats after simulated microgravity with and without daily -Gx gravitation. *J Appl Physiol* 2008; 105: 1134-1145.
14. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. San Diego, Academic Press, 1998.
15. Halloran BP, Bikle DD, Cone CM, Morey-Holton E. Glucocorticoids and inhibition of bone formation induced by skeletal unloading. *Am J Physiol* 1988; 255: E875-E879.
16. Steffen JM, Musacchia XJ. Disuse atrophy, plasma corticosterone, and muscle glucocorticoid receptor levels. *Aviat Space Environ Med* 1987; 58: 996-1000.
17. Fyhrquist F, Tikkanen I, Linkola J. Plasma vasopressin concentration and renin in the rat: effect of hydration and hemorrhage. *Acta Physiol Scand* 1981; 113: 507-510.
18. Torp M, Brond L, Hadrup N, *et al*. Losartan decreases vasopressin-mediated cAMP accumulation in the thick ascending limb of the loop of Henle in rats with congestive heart failure. *Acta Physiol* (*Oxf*) 2007; 190: 339-350.
19. Ding JM, Carver WC, Terracio L, Buggy J. Proto-oncogene c-fos and the regulation of vasopressin gene expression during dehydration. *Brain Res Mol Brain Res* 1994; 21: 247-255.
20. Drummer C, Heer M, Joosten M, *et al*. Regulation and distribution of body fluid during a 6-day head-down tilt study in a randomized cross-over design. *J Gravit Physiol* 2000; 7: P187-P188.
21. Nakamitsu S, Sagawa S, Miki K, *et al*. Effect of water temperature on diuresis-natriuresis: AVP, ANP, and urodilatin during immersion in men. *J Appl Physiol* 1994; 77: 1919-1925.
22. Drummer C, Gerzer R, Heer M, Molz B, *et al*. P Effects of an acute saline infusion on fluid and electrolyte metabolism in humans. *Am J Physiol* 1992; 262: F744-F754.
23. Drummer C, Gerzer R, Baisch F, Heer M. Body fluid regulation in micro-gravity differs from that on Earth: an overview. *Pflugers Arch* 2000; 441: R66-R72.
24. Leach CS. Medical results from STS 1-4: analysis of body fluids. *Aviat Space Environ Med* 1983; 54: S50-S54.
25. Leach CS, Alfrey CP, Suki WN, *et al*. Regulation of body fluid compartments during short-term spaceflight. *J Appl Physiol* 1996; 81: 105-116.
26. Mueller PJ, Sullivan MJ, Grindstaff RR, Cunningham TJ, Hasser EM. Regulation of plasma vasopressin and renin activity in conscious hindlimb-unloaded rats. *Am J Physiol Regul Integr Comp Physiol* 2006; 291: R46-R52.
27. Wade CE, Morey-Holton E. Alteration of renal function of rats following spaceflight. *Am J Physiol* 1998; 275: R1058-R1065.
28. Sullivan MJ, Hasser EM, Moffitt JA, Bruno SB, Cunningham TJ. Rats exhibit aldosterone-dependent sodium appetite during 24 h hindlimb unloading. *J Physiol* 2004; 557: 661-670.
29. Brady HR, Singer GG. Acute renal failure. *Lancet* 1995; 346: 1533-1540.
30. Smith SM, Krauhs JM, Leach CS. Regulation of body fluid volume and electrolyte concentrations in spaceflight. *Adv Space Biol Med* 1997; 6: 123-165.
31. Chowdhury P, Soulsby ME, Scott JL. Effects of aminoguanidine on tissue oxidative stress induced by

hindlimb unloading in rats. *Ann Clin Lab Sci* 2009; 39: 64-70.
32. Wong NL, Tsui JK. Angiotensin II upregulates the expression of vasopressin V2 mRNA in the inner medullary collecting duct of the rat. *Metabolism* 2003; 52: 290-295.
33. Wang W, Li C, Summer S, Falk S, Schrier RW. Interaction between vasopressin and angiotensin II in vivo and in vitro: effect on aquaporins and urine concentration. *Am J Physiol Renal Physiol* 2010; 299: F577-F584.
34. Sansoe G, Aragno M, Smedile A, Rizzetto M, Rosina F. Solute-free water retention in preascitic cirrhotic rats following intravenous water loading. *J Physiol Pharmacol* 2009; 60: 111-117.
35. Boczek-Leszczyk E, Stempniak B, Juszczak M. Vasopressin release from the rat hypothalamo-neurohypophysial system: effects of gonadotrophin-releasing hormone (GnRH), its analogues and melatonin. *J Physiol Pharmacol* 2010; 61: 459-466.
36. Gazenko OG, Natochin V, Ilyin AY, *et al*. Fluid-electrolyte metabolism and renal function of white rats in experiments aboard cosmos biosatellites. *Aviat Space Environ Med* 1984; 55: 685-691.
37 Gazenko OG, Shulzhenko EB, Egorov AD. Cardiovascular changes in prolonged space flights. *Acta Physiol Pol* 1986; 37: 53-68.
38. Natochin VY, Grigoriev AI, Noskov VB, *et al*. Mechanism of postflight decline in osmotic concentration of urine in cosmonauts. *Aviat Space Environ Med* 1991; 62: 1037-1043.
39. Watenpaugh DE, Hargens AR. The cardiovascular system in microgravity. In: Handbook of Physiology: Environmental Physiology, MJ Fregly, CM Blatteis (eds), Vol 1, Section 4. Bethesda MD, American Physiology Society, 1996, pp. 631-674.
40. Bullitt E. Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. *J Comp Neurol* 1990; 296: 517-530.

Received: September 5, 2011
Accepted: January 31, 2012

Author's address: Dr. Min-Soo Kwon, Assistant Professor, Department of Pharmacology, School of Medicine, CHA University, Bundang-gu, Seongnam, Gyeonggi-do, South Korea; Phone: +82-31-725-8303; E-mail: minsoo100@cha.ac.kr