Osmoregulation of vasopressin release and gene transcription under acute and chronic hypovolemia in rats

Noriko Kondo, Hiroshi Arima, Ryouichi Banno, Shinobu Kuwahara, Ikuko Sato, and Yutaka Oiso

Department of Metabolic Diseases, Field of Internal Medicine, Nagoya University
Graduate School of Medicine, Nagoya 466-8550, Japan

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Kondo, Noriko, Hiroshi Arima, Ryouichi Banno, Shinobu Kuwahara, Ikuko Sato, and Yutaka Oiso. Osmoregulation of vasopressin release and gene transcription under acute and chronic hypovolemia in rats. Am J Physiol Endocrinol Metab 286: E337–E346, 2004.—Although acute decreases in plasma volume are known to enhance the osmotically induced arginine vasopressin (AVP) release, it is unclear whether there is such interaction at the level of gene transcription. It also remains to be established how sustained changes in plasma volume affect the osmoregulation. In this study, we examined how acute and chronic decreases in blood volume affected the osmoregulation of AVP release and gene transcription in rats. Acute hypovolemia was induced by intraperitoneal injection of polyethylene glycol (PEG), and chronic hypovolemia was induced by 3 days of water deprivation (WD) or 12 days of salt loading (SL). Rats were injected with isotonic or hypertonic saline, and plasma AVP levels and AVP heteronuclear (hn)RNA expression in the supraoptic and paraventricular nuclei, an indicator of gene transcription, were examined in relation to plasma osmolality in each group. Plasma AVP levels were correlated with plasma Na levels in all groups. Whereas the regression lines relating plasma AVP to Na were almost identical among control, WD, and SL groups, the thresholds of plasma Na for AVP release were significantly increased only in the PEG group. AVP hnRNA levels were also correlated with plasma Na levels in control and PEG groups, and the thresholds were significantly decreased in the PEG group. In contrast, there was no significant correlation of AVP hnRNA and plasma Na levels in WD and SL groups. Thus it was demonstrated that acute and chronic reduction in plasma volume affected the osmoregulation of AVP release and gene transcription in different ways.

heteronuclear RNA; water deprivation; salt loading; polyethylene glycol; hypertonic saline

ARGININE VASOPRESSIN (AVP), an antidiuretic hormone, is synthesized in magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus, transported axonally, and released into the systemic circulation from the nerve terminals in the posterior pituitary (6, 27). Increases in plasma osmolality are sensed in the osmoreceptor, which is believed to exist in the organum vasculosum of the lamina terminalis (7), and induce AVP release as well as AVP gene transcription in the SON and PVN (4). The osmoregulation is so precise that changes of only 1% in plasma osmolality could significantly affect AVP release and gene transcription (4). On the other hand, reductions in plasma volume detected in the cardiac atria (10, 14, 37) increase AVP release and its gene transcription when the changes in plasma volumes are >10% (16, 30). Thus AVP magnocellular neurons are regulated by changes in plasma osmolality and volume under physiological conditions, and AVP release and gene transcription are tightly coupled in response to acute changes in plasma osmolality or volume.

There is an interaction between osmo- and volume regulation of AVP release (8, 15, 22, 23, 25, 28, 33, 38). Acute changes in plasma volume have been shown to significantly affect the set point and/or sensitivity of AVP release in response to changes in plasma osmolality (8, 22, 23, 25, 28). Such interaction could be important, because the increased plasma AVP levels due to decrease in effective plasma volume might be responsible for hyponatremia in some clinical disorders such as cirrhosis and congestive heart failure (1, 29, 34). However, it should be noted that changes in plasma volume in these conditions were usually chronic and could have different effects on osmoregulation from acute hypovolemia. Several approaches have been used so far in an effort to clarify the osmoregulation under conditions in which plasma volume was chronically decreased. Although there is a study suggesting that chronic changes in plasma volume could increase the sensitivity of AVP release to changes in plasma osmolality (36), other studies (13, 26) showed that the sensitivity of AVP release under chronic hypovolemia was similar to that in normovolemic control. Even in the latter studies, however, the threshold of plasma osmolality for AVP release was shifted to the left, as in acute hypovolemia. Thus it is unclear whether or not volume regulation of AVP release would be reset under chronic hypovolemia so that an organism could adapt to changes in plasma volume. It also remains to be established how acute or chronic changes in plasma volume affect the osmoregulation of AVP gene transcription in the magnocellular neurons.

To better understand the interaction between osmo- and volume regulation of AVP neurons, we examined osmoregulation of AVP release and gene transcription in the SON and PVN under acute and chronic hypovolemia in rats. To induce chronic hypovolemia, we employed water deprivation and salt loading, both of which are known to decrease plasma volume substantially (3, 40).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (body wt 250–300 g; Chubu Science Materials, Nagoya, Japan) were housed two per plastic cage.

Address for reprint requests and other correspondence: H. Arima, Dept. of Metabolic Diseases, Field of Internal Medicine, Nagoya Univ. Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan (E-mail: arima105@med.nagoya-u.ac.jp).

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under controlled conditions (23.0 ± 0.5°C; lights on, 0900–2100) and provided with standard rat chow ad libitum. All procedures were performed in accordance with institutional guidelines for animal care at Nagoya University Graduate School of Medicine.

**Osmoregulation of AVP release and gene transcription under acute hypovolemia.** Polyethylene glycol (PEG, MW 3,000; Wako Pure Chemical Industries, Osaka, Japan) is known to reduce plasma volume without altering plasma osmolality (8). To induce acute hypovolemia, PEG dissolved in isotonic saline (30% wt/vol) was injected (2% body wt ip) intraperitoneally to rats 90 min before decapitation. To examine osmoregulation of AVP release and gene transcription under acute hypovolemia, hypertonic (900 mosmol/kg) saline was further injected (2% body wt ip) 30 min before decapitation. Isotonic (290 mosmol/kg) saline was injected (2% body wt ip) as a control for PEG and hypertonic saline injection. The number of rats in each group was 7–9.

**Osmoregulation of AVP release and gene transcription under chronic hypovolemia.** Rats were divided into three groups: 1) control, 2) deprived of water for 3 days, and 3) salt loaded (2% NaCl in drinking water) for 12 days. Rats were injected (2% body wt ip) with either isotonic (290 mosmol/kg) or hypertonic (900 mosmol/kg) saline 30 min before decapitation. The number of rats in each group was 6–8.

**Estimation of blood volume.** The levels of total protein (TP) in plasma were used to estimate acute changes in plasma volume in PEG experiments (33). For water-deprived or salt-loaded rats, plasma volume was directly determined with Evans blue dye (EB), because plasma TP levels could be altered by factors other than plasma volume under chronic dehydration (9). Rats were injected intravenously with 0.5 ml of 1% EB (Sigma Chemical, St. Louis, MO) after anesthesia with diethyl ether in separate experiments (20). One minute later, the rats were decapitated, and blood samples were collected and centrifuged immediately at 4°C. The optical density (OD) of each plasma sample was measured at 620 nm (OD 620). In each sample, the concentration of EB was calculated from the corrected OD 620 with a linear standard curve for EB (range: 0.0–2.0 mg/dl). Finally, plasma volume was calculated as plasma volume (ml) = 5 (mg) × 100 / concentration of EB (mg/dl)

The number of rats in each group was 12.

**Measurement of plasma AVP, sodium, TP, and pituitary AVP contents.** After decapitation, trunk blood was collected into chilled tubes containing EDTA (potassium salt). After immediate separation of blood contents.

| Table 1. Changes in plasma Na and total protein levels |
|----------------|----------------|----------------|----------------|
|                | Control IS     | Control HS    | PEG IS         |
| Plasma TP, g/dl| 5.7±0.1        | 5.8±0.1       | 7.4±0.1*       |
| Plasma Na, mmol/l| 140.9±0.5    | 147.0±0.5*    | 139.3±0.5      |
| Plasma volume, %| 100.0±1.0      | 98.3±1.5      | 76.4±1.5*      |

Values are means ± SE. Plasma total protein (TP) levels were increased by ip injection (2% body wt) of polyethylene glycol (PEG) compared with control injection with isotonic saline (IS). Plasma Na levels were increased significantly by ip injection (2% body wt) of hypertonic saline (HS; 900 mosmol/kg) compared with control IS injection. Intraperitoneal injection of PEG produced a reduction in plasma volume of ~23% compared with control. *P < 0.05 vs. control.
at 4°C, AVP was extracted through a Sep-Pak C18 Cartridge (Waters Associates, Milford, MA) and measured with a highly sensitive RIA kit (AVP-RIA kit, kindly provided by Mitsubishi Chemical, Tokyo, Japan). The sensitivity of the assay for AVP was 0.063 pg/tube (0.17 pg/ml), with <0.01% cross-reactivity with oxytocin (24). Plasma sodium (Na) and TP were measured with an autoanalyzer (Hitachi, Tokyo, Japan).

Pituitary glands were removed immediately after decapitation and then kept in acetone at −20°C. Each pituitary gland was homogenized and stirred in 0.1 N HCl at 4°C for 24 h. After centrifugation, the supernatants were diluted and assayed for AVP contents.

In situ hybridization. The rat AVP intronic probe (kindly provided by Dr. Thomas Sherman, Georgetown University, Washington, DC) was a 735-bp fragment of intron 1 of the rat AVP gene subcloned into pGEM-3 and linearized by HindIII. Highly specific antisense probes were synthesized using 55 Ci [35S]UTP and 171 Ci [35S]CTP (PerkinElmer Life Sciences, Natick, MA), the Riboprobe Combination System (Promega, Madison, WI), 15 U RNasin, 1 μg linearized template, and 15 U of T7 RNA polymerase. After 60 min of incubation at 42°C, the cDNA template was digested with DNase for 10 min at 37°C. Radiolabeled RNA products were purified using quick-spin columns (Roche Diagnostics, Indianapolis, IN), precipitated with ethanol, and resuspended in 100 μl of 10 mM Tris-HCl, pH 7.5, containing 20 mM DTT.

The collected brains were stored at −80°C until sectioning for in situ hybridization. Five sets of sequential coronal sections of 12 μm were cut in a cryostat, thaw-mounted onto poly-L-lysine-coated slides, and stored at −80°C until hybridization. After thawing at room temperature, sections were fixed in 4% formaldehyde in PBS for 5 min and acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine-0.9% NaCl, pH 8, for 10 min at room temperature. Sections were then dehydrated in 70, 80, 95, and 100% ethanol, delipidated in chloroform, and hybridized overnight at 55°C with 2 × 106 counts/min of 35S-labeled probes in 95 μl of hybridization buffer (50% formamide, 200 mM NaCl, 2.5 mM EDTA, 10% dextran sulfate, 250 μg/ml yeast tRNA, 50 mM DTT, and 1% Denhardt’s solution). At the end of incubation, sections were subjected to consecutive washes in 4X standard saline citrate (SSC) for 15 min at room temperature and 50% formamide-250

Fig. 3. Effects of acute hypovolemia and ip injection of hypertonic saline on AVP heteronuclear (hn)RNA gene expression in the supraoptic nucleus (SON, A) and paraventricular nucleus (PVN, B). Rats were injected ip (2% body wt) with either IS (290 mosmol/kg) or PEG 90 min before decapitation, and further injected ip (2% body wt) with either IS or HS (900 mosmol/kg) 30 min before decapitation. Values are means ± SE of no. of rats/group indicated above each bar. Comparison between groups was performed by one-way ANOVA followed by Fisher’s PLSD test. *P < 0.01 vs. control rats injected with IS.

Fig. 4. Regression analyses of plasma Na and AVP gene transcription in the SON (A and B) and PVN (C and D) in control (A and C) and PEG-injected rats (B and D). Plasma Na and AVP gene transcription in the SON and PVN showed positive correlation in both groups. Thresholds of plasma Na for AVP gene transcription in the SON and PVN were significantly decreased in PEG-injected rats compared with control. Control data are represented as dotted lines in B and D.
mM NaCl containing DTT for 15 min at 60°C. After treatment with RNase A (20 μg/ml) for 30 min at 37°C, sections were washed with 2× SSC, 1× SSC, and 0.5× SSC for 5 min each at room temperature, followed by washes with 0.1× SSC containing DTT for 15 min at 50°C, and with 0.1× SSC to cool at room temperature and with 70% ethanol for 15 s. For analysis of AVP heteronuclear (hn)RNA, sections from each experimental group were placed in the same X-ray cassettes and exposed to Kodak BioMax MR films (Kodak, Rochester, NY) for 24 h. The ODs of the autoradiographs were quantified using a computer image analysis system (Imaging Research, Ontario, Canada) and the public domain NIH Image program (developed at the National Institutes of Health and made available for downloading at http://rsb.info.nih.gov/nih-image).

Changes in AVP hnRNA levels were quantified by measurements of the integrated OD (OD × area) of the film images. The total sum of OD signals of AVP hnRNA in the bilateral SON or PVN in the sections from each rat was used in the analysis. The mean values of control AVP hnRNA expression levels were expressed as 100%.

Slides containing PVN were dipped in nuclear Kodak NTB2 emulsion and exposed for 2 days. To assist cellular localization of the hybridized signals, the emulsion-dipped sections were stained with cresyl violet. The medial parvocellular AVP neurons in the PVN were differentiated from magnocellular neurons on the basis of their overall size, their relatively low level of AVP expression, and their small, dense-staining nuclei (11, 19).

Statistics. Statistical significance of the differences between groups was calculated by one-way ANOVA followed by Fisher’s protected least significant difference (PLSD) test. Results are expressed as means ± SE, and differences were considered significant at \( P < 0.05 \). Linear regression analyses were performed using the least square method. Differences between regression lines were evaluated statistically by analysis of covariance.

RESULTS

Osmoregulation of AVP release and gene transcription under acute hypovolemia. In rats injected with isotonic saline 90 min before decapitation, the additional intraperitoneal injection of hypertonic saline increased plasma Na levels (Table 1) and produced significant rises in plasma AVP and AVP hnRNA expression in the SON and PVN compared with control (Fig. 1, plasma AVP, 7.89 ± 0.54 vs. control 1.10 ± 0.25).

Table 2. Changes in plasma Na, body wt, pituitary AVP contents, and plasma volume in control, WD, and SL rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control+HS</th>
<th>WD</th>
<th>WD+HS</th>
<th>SL</th>
<th>SL+HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na, mmol/l</td>
<td>139.9±0.6</td>
<td>147.3±0.5*</td>
<td>140.5±1.0</td>
<td>151.8±0.5*</td>
<td>141.0±1.0</td>
<td>149.0±1.3*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>320.7±3.3</td>
<td>324.7±2.9*</td>
<td>294.7±2.9*</td>
<td>267.3±5.6*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pituitary AVP contents, μg/pituitary</td>
<td>1.84±0.07</td>
<td>ND</td>
<td>0.98±0.05*</td>
<td>ND</td>
<td>0.33±0.08*</td>
<td>ND</td>
</tr>
<tr>
<td>Plasma volume, %</td>
<td>100.0±2.5</td>
<td>78.4±2.0*</td>
<td>ND</td>
<td>ND</td>
<td>64.1±2.7*</td>
<td>ND</td>
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</table>

Values are means ± SE. AVP, arginine vasopressin. Plasma Na levels were increased significantly by ip injection (2% body wt) of HS (900 mosmol/kg) compared with control IS rats. In separate experiments, rats were injected iv with 0.5 ml of 1% Evans blue dye for estimation of changes in plasma volume due to 3 days of water deprivation (WD) or 12 days of salt loading (SL). *\( P < 0.05 \) vs. each control. ND, not determined.
0.21 pg/ml, \( P < 0.01 \); see also Fig. 3A. AVP hnRNA levels in the SON, 258.2 ± 16.5 vs. control 100.0 ± 6.3%, \( P < 0.01 \); Fig. 3B, AVP hnRNA levels in the PVN, 237.4 ± 16.4 vs. control 100.0 ± 3.3%, \( P < 0.01 \). As reported previously (4), microscopic analyses of emulsion-dipped slides revealed that, whereas AVP hnRNA was expressed robustly in the magnocellular neurons, virtually no signals in the parvocellular neurons were recognized in the PVN even after intraperitoneal injection of hypertonic saline (data not shown), indicating that changes in AVP hnRNA levels in the PVN reflect changes in the magnocellular neurons. The regression analyses (31) between plasma Na and plasma AVP or AVP hnRNA levels showed a positive correlation in rats injected with isotonic saline 90 min before decapitation [Fig. 2A, plasma AVP = 0.959 (plasma Na = 139.3), \( r = 0.895 \); and see Fig. 4A, AVP hnRNA levels in the SON = 12.823 (plasma Na = 136.4), \( r = 0.926 \); Fig. 4C, AVP hnRNA levels in the PVN = 15.975 (plasma Na = 136.0), \( r = 0.932 \)].

The intraperitoneal injection of PEG produced a reduction in plasma volume of ~23% compared with control (Table 1), accompanied by significant increases in plasma AVP as well as AVP hnRNA levels in the SON and PVN (Figs. 1 and 3). In rats injected with PEG, the additional intraperitoneal injection of hypertonic saline (2% body wt) of hypertonic (900 mosmol/kg) saline on pituitary AVP contents. As there were no significant differences in pituitary AVP contents between rats injected with isotonic and slopes of these graphs were not significantly different between control and PEG groups, the thresholds of plasma Na for AVP release and gene transcription shown by the x-intercepts were significantly lower in PEG than in control \( (P < 0.05; \text{ Figs. 2 and 4}) \). Representative autoradiographs are shown in Fig. 5.

### Osmoregulation of AVP release and gene transcription under chronic hypovolemia

Changes in plasma Na, body weight, pituitary AVP contents, and plasma volume during chronic dehydration were shown in Table 2. In separate experiments, we examined the effects of intraperitoneal injection (2% body wt) of hypertonic (900 mosmol/kg) saline on pituitary AVP contents. As there were no significant differences in pituitary AVP contents between rats injected with isotonic and

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\text{Plasma AVP} = 0.959 \times \text{plasma Na} - 139.3
\]

\[
\text{AVP hnRNA} = 12.823 \times \text{plasma Na} - 136.4
\]

\[
\text{AVP hnRNA} = 15.975 \times \text{plasma Na} - 136.0
\]

\[
\text{Thresholds of plasma Na for AVP release were not significantly different among the 3 groups. The slope in A is shown as dotted lines in B and C.}
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**Fig. 6. Effects of chronic dehydration and ip injection of HS on AVP release.** Rats were divided into 3 groups: control, deprived of water for 3 days (WD), and salt loaded (2% NaCl-drinking) for 12 days (SL). Rats were injected (2% body wt) ip with either IS (290 mosmol/kg) or HS (900 mosmol/kg) 30 min before decapitation. Values are means ± SE of no. of rats/group indicated above each bar. Comparison between groups was performed by one-way ANOVA followed by Fisher’s PLSD test. *\( P < 0.01 \) vs. control rats injected with IS.

**Fig. 7. Regression analyses of plasma Na and AVP release in control rats (A), rats deprived of water for 3 days (WD, B), and rats salt loaded for 12 days (SL, C).** Plasma Na and AVP levels showed a positive correlation in all groups. Thresholds of plasma Na for AVP release were not significantly different among the 3 groups. The slope in A is shown as dotted lines in B and C.
hypertonic saline (data not shown), pituitary AVP contents were examined only in rats injected with isotonic saline in the following experiments and shown in Table 2. Although there were no significant differences in plasma Na levels between control, water-deprived, and salt-loaded rats injected with isotonic saline (Table 2), plasma Na levels were significantly higher in water-deprived and salt-loaded than in control rats when they were not injected with saline (control, 140.0 ± 0.2 vs. water-deprived rats, 143.6 ± 0.3; salt-loaded rats, 142.0 ± 0.5 mmol/l, P < 0.05 for each). This suggested that intraperitoneal injection of isotonic saline decreased plasma Na levels in dehydrated rats but not in euhydrated rats. The reduction in plasma volume was 36% in salt-loaded rats and 22% in water-deprived rats (Table 2).

The levels of plasma AVP in rats injected with isotonic saline were significantly higher in water-deprived and salt-loaded rats than in control rats (Fig. 6). The intraperitoneal injection of hypertonic saline produced significant rises in plasma AVP as well as plasma Na levels in all groups (Table 2, Fig. 6). The regression analyses between plasma Na and plasma AVP showed a positive correlation in all groups (Fig. 7). There were no significant differences in the sensitivity or threshold of plasma Na for AVP release among control, water-deprived, or salt-loaded rats. The levels of AVP hnRNA in the SON and PVN in rats injected with isotonic saline were significantly higher in water-deprived and salt-loaded rats than in control rats (Fig. 8, A and B), consistent with previous results showing that AVP hnRNA levels were increased after salt loading (12). The intraperitoneal injection of hypertonic saline produced significant rises in AVP hnRNA levels in the SON and PVN in control rats (Fig. 8, A and B), and regression analyses showed a positive correlation (Fig. 9, A and D). On the other hand, hypertonic saline injection did not significantly increase AVP hnRNA levels in the SON or PVN in water-deprived or salt-loaded rats (Fig. 8, A and B). Regression analyses did not show a positive correlation between plasma Na and AVP hnRNA levels in water-deprived (Fig. 9, B and E) or salt-loaded rats (Fig. 9, C and F). Representative autoradiograms are shown in Fig. 10. Analyses of emulsion-dipped slides showed that AVP hnRNA was expressed in the magnocellular neurons but not in the parvocellular neurons in the PVN in any group of dehydrated rats (Fig. 11).

**DISCUSSION**

In the present study, we sought to elucidate the osmoregulation of AVP release and the gene transcription in the SON and PVN under acute and chronic hypovolemia. To estimate gene transcription, we measured AVP hnRNA, which was previously shown to be a sensitive indicator for gene transcription (12). Our data clearly showed that acute reduction in plasma volume decreased the set point for AVP hnRNA expression in the magnocellular neurons as well as AVP release, whereas chronic reduction in plasma volume did not affect osmoregulation of AVP release. Furthermore, it was demonstrated that AVP hnRNA expression did not respond to increases in plasma osmolality under chronic dehydration.

We first examined the osmoregulation of AVP release under acute hypovolemia when the plasma volume was decreased 23%. Unlike previous studies (8, 27), differences in the sensitivities for AVP release between control and hypovolemic rats did not reach significance. In the present study, we injected rats with isotonic or 900 mosmol/kg saline to analyze the sensitivity. Whereas plasma Na levels ranged between 137 and 150 mmol/l, most values clustered around either 140 or 148 mmol/l, which might make it difficult to see the changes in the sensitivity. Alternatively, because it was shown that the sensitivity could be increased if the volume depletion by PEG injection exceeded a certain point (33), changes in volume by PEG injection might not be enough in the present study. On the other hand, our data showed that the set point for AVP release was significantly decreased, as shown in previous studies (8, 23, 25, 28), indicating that the responses to AVP release in relation to changes in plasma osmolality are more robust under acute hypovolemia. We then examined AVP hnRNA expression in the SON and PVN in the same experiment and found that the set point for AVP hnRNA expression was also significantly decreased. These data suggest that the interaction between...
osmo- and volume regulation exists at the levels of not only release (8, 15, 22, 23, 25, 28, 33, 38) but also gene transcription, and they provide further convincing evidence that AVP release and gene transcription are tightly coupled in response to acute changes in plasma osmolality and/or volume (4, 16).

In the present study, we employed 3 days of water deprivation and 12 days of salt loading to induce chronic dehydration. Decrease in body weight in these regimens could be due not only to dehydration but also to decrease in food intake (9), although food restriction per se is not shown to affect AVP release (17). Plasma volume was decreased 22 and 36% in water-deprived and salt-loaded rats, respectively, amounts that were similar to, or even greater than, changes induced by PEG. Furthermore, pituitary AVP contents, which might affect osmoregulation of AVP release (21), were significantly decreased in water-deprived and salt-loaded rats. Nevertheless, our data showed that the set point and sensitivity for AVP release were not affected under chronic dehydration, indicating that a chronic decrease in plasma volume, in contrast to acute hypovolemia, had no significant effect on osmoregulation of AVP release. This is not consistent with results from a previous study (36) in which the slope showing the relationship between plasma Na and AVP was steeper in water-deprived rats than in euhydrated rats. However, in that study, euvhydrated rats were injected with hypertonic saline to analyze the correlation, whereas water-deprived rats were not; therefore, the differences in the slope might simply reflect the acute volume expansion in hydrated rats. The adaptation of AVP release to chronic changes in plasma volume has also been suggested in other models of chronic hypovolemia induced by repeated injection of furosemide in rats (13) and by peritoneal dialysis in anephric dogs (26). In these models, however, the set points of AVP release were still shifted to the left, as in acute hypovolemia (13, 26). Indeed, whereas plasma Na levels were not different between groups after injection of isotonic saline, the plasma AVP levels in water-deprived and salt-loaded rats were significantly higher than in control rats, even in the present study. Thus, although it is clearly demonstrated that chronic volume depletion has less effect on osmoregulation than acute volume depletion, the adaptation under chronic dehydration might still be incomplete.

Fig. 9. Regression analyses of plasma Na and AVP gene transcription in the SON (A, B, and C) and PVN (D, E, and F) in control (A and D), WD (B and E), and SL (C and F) rats. Plasma Na and AVP levels showed positive correlation in the control condition but not in chronic dehydration (B, C, E, and F).
The discrepancy in the regulation of AVP release under acute and chronic hypovolemia could be relevant to the hemodynamics and water balance in clinical conditions. AVP acts not only via the v2 receptor in the kidney to reabsorb water but also via the v1 receptor to construct the artery (27). Acute reductions in plasma volume, such as severe hemorrhage, could cause a reduction in blood pressure (16). Under such conditions, preventing decreases in blood pressure would be more important for an organism than the precise osmoregulation of AVP release. In contrast, it would be beneficial for an organism to maintain precise osmoregulation in conditions in which plasma volume was gradually decreased and blood pressure was not decreased. Thus, volume regulation of AVP release would be adapted and reset under chronic hypovolemia.

Our data showed that, after rats were exposed to chronic hyperosmolality and hypovolemia, AVP hnRNA expression in the SON or PVN did not increase significantly in response to the increases in plasma Na levels, and that AVP hnRNA levels were not correlated to plasma Na levels. This is in marked contrast to the precise osmoregulation of AVP release maintained under chronic dehydration. Although it is not clear why AVP gene transcription became refractory to changes in plasma osmolality in the present study, it is possible that signaling pathways involved in AVP gene transcription were already so fully stimulated under chronic dehydration that acute osmotic stimulus did not cause further stimulation. In any case, it is demonstrated that, although AVP release and synthesis are tightly coupled during acute osmotic stimuli, they are uncoupled under chronic dehydration, as has been suggested in dehydration-rehydration models (5, 32, 39).

Salt loading, intraperitoneal injection of hypertonic saline, and acute hypovolemia are reported to activate parvocellular neurons, as evidenced by the induction of AVP and CRH gene expression (2, 3, 18, 35), suggesting that these regimens are physical and/or psychological stressors. Indeed, intraperitoneal injection of hypertonic saline has been used not only as an osmotic stimulus but also as a painful stress (18, 19). It should be noted, however, that AVP mRNA or hnRNA expression in the parvocellular neurons was much lower than in the magnocellular neurons in the PVN, even in these conditions, and longer exposure is necessary to see the expression in the parvocellular neurons. In addition, the dose of hypertonic saline employed in this study (900 mosmol/kg) is much lower than that (1.5 M) in previous studies (18, 19). Although the regimens employed might also activate parvocellular neurons, microscopic analysis of emulsion-dipped slides in this study revealed that changes in AVP hnRNA expression in the parvocellular neurons had little effect, if any, on the analyses of AVP expression in the PVN.

In conclusion, we showed that acute reduction in plasma volume significantly affected the osmoregulation of AVP hnRNA expression in the SON and PVN, as well as AVP release, in such a way that the set points were decreased. Furthermore, our data demonstrated that the osmoregulation of AVP release was not affected by a chronic decrease in plasma volume, whereas AVP gene transcription did not...
Fig. 11. AVP hnRNA expression in PVN in emulsion-dipped slides of control (A) and both WD and HS (B) rats. Parvocellular AVP neurons in the PVN were differentiated from magnocellular neurons on the basis of their overall size, their relatively low level of AVP expression, and their small, dense-staining nuclei. Arrowheads, magnocellular neurons; arrows point to parvocellular neurons.

respond to an acute increase in plasma osmolality under chronic dehydration.

GRANTS

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REFERENCES


35. Tanimura SM, Sanchez-Watts G, and Watts AG. Peptide gene activation, secretion, and steroid feedback during stimulation of rat neuroendo-


