Vasopressin gene transcription increases in response to decreases in plasma volume, but not to increases in plasma osmolality, in chronically dehydrated rats

Masayuki Hayashi, Hiroshi Arima, Motomitsu Goto, Ryouichi Banno, Minemori Watanabe, Ikuko Sato, Hiroshi Nagasaki, and Yutaka Oiso

Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya, Japan

Submitted 8 April 2005; accepted in final form 31 August 2005

Hayashi, Masayuki, Hiroshi Arima, Motomitsu Goto, Ryouichi Banno, Minemori Watanabe, Ikuko Sato, Hiroshi Nagasaki, and Yutaka Oiso. Vasopressin gene transcription increases in response to decreases in plasma volume, but not to increases in plasma osmolality, in chronically dehydrated rats. Am J Physiol Endocrinol Metab 290: E213–E217, 2006. First published September 6, 2005; doi:10.1152/ajpendo.00158.2005.—The synthesis of arginine vasopressin (AVP) in the magnocellular neurons of the supraoptic (SON) and paraventricular nuclei (PVN) is physiologically regulated by plasma osmolality and volume. To clarify how the regulation of AVP gene transcription is affected by chronic dehydration, we examined changes in the transcriptional activities of AVP gene by plasma osmolality and volume in both euhydrated and dehydrated conditions. Euhydrated rats had free access to water, whereas dehydrated rats had been deprived of water for 3 days before experiments. Rats in both conditions were subjected to acute hypertonic stimuli or hypovolemia, and changes in AVP heteronuclear (hn)RNA levels, an indicator of gene transcription, in the SON and PVN were examined with in situ hybridization. The intraperitoneal (ip) injection (2% body wt) of hypertonic (1.5 M) saline increased plasma Na levels by ~40 meq/l in both euhydrated and dehydrated conditions. However, expression levels of AVP hnRNA in the SON and PVN were increased only in euhydrated, not dehydrated, rats. On the other hand, ip injection of polyethylene glycol decreased the plasma volume by ~16–20%, and AVP hnRNA levels in the SON and PVN were significantly increased in both conditions. Thus it is demonstrated that signaling pathways regulating AVP gene transcription in the magnocellular neurons were completely refractory to acute osmotic stimuli under the chronic dehydration and that AVP gene transcription could probably respond to acute hypovolemia through different intracellular signal transduction pathways from those for osmoregulation.

dehydration; hyperosmolality; hypovolemia

ARGININE VASOPRESSIN (AVP) is synthesized in magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) in the hypothalamus, transported axonally, and released into the systemic circulation from the nerve terminals in the posterior pituitary (2). The release and gene transcription of AVP are regulated physiologically by plasma osmolality and blood volume (1, 4, 9, 14, 17). Changes in plasma osmolality are sensed at the osmoreceptors, which are believed to exist in the organum vasculosum of the lamina terminalis (3), and the signals are then transferred to the magnocellular neurons (2, 3). The osmoregulation of magnocellular AVP systems is so precise that only a 1% change in plasma osmolality could affect both AVP release and gene transcription (1). On the other hand, changes in blood volume are detected at volume receptors located in the cardiac atria, and the signals are transferred through the vagal nerves to the nucleus solitarius in the brain stem, from which postsynaptic pathways project to the magnocellular AVP neurons (2, 16). In contrast to the osmoregulation, AVP release and gene transcription are significantly increased only when decreases in plasma volume exceed 10–20% (4, 8, 9, 15, 17).

Although AVP release and gene transcription are tightly coupled in most cases (1, 8), this is not true under chronic dehydration. We previously reported that, although AVP release is precisely regulated by plasma Na levels, the levels of AVP heteronuclear (hn)RNA, the first transcript and a sensitive indicator of transcription (6), were not affected significantly in the SON and PVN by changes in plasma Na levels between 140 and 150 meq/l in rats deprived of water for 3 days (9). These data indicate that, unlike AVP release, the precise osmoregulation of AVP gene transcription was lost under chronic dehydration. One possible explanation for these findings is that, although information on changes in plasma osmolality was indeed transferred to the magnocellular AVP neurons, as reflected by increases in AVP release, AVP gene transcription may have reached maximal levels and therefore did not increase in response to acute osmotic stimulus under chronic dehydration. If so, AVP gene transcription might not respond to any kind of stimuli, including huge increases in plasma osmolality and decreases in plasma volume. It is also possible that AVP gene transcription is refractory only to increases in plasma osmolality yet still responds to decreases in plasma volume in cases where intracellular signal transduction pathways are different between osmo- and volume regulation of AVP gene transcription under the chronic dehydration.

To address these issues, in the present study we examined the effects of the osmotic stimuli (~40 meq/l increases in plasma Na levels) as well as hypovolemia on AVP gene transcription in the SON and PVN in chronically dehydrated rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (250–300 g body wt; Chubu Science Materials, Nagoya, Japan) were housed two per plastic cage under controlled conditions (23.0 ± 0.5°C, lights on from 0900 to 2100). Until the experiments, the euhydrated rats were provided with

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
standard rat chow and water ad libitum, whereas the dehydrated rats were provided with chow ad libitum but deprived of water for 3 days. After intraperitoneal (ip) injection, rats were not allowed access to chow or water. All procedures were performed in accordance with the institutional guidelines for animal care at Nagoya University Graduate School of Medicine and were approved by The Animal Experimentation Committee.

**Experiment 1:** effects of acute osmotic stimuli on AVP release and gene transcription under euhydrated and dehydrated conditions. Rats in both euhydrated and dehydrated groups were injected ip (2% body wt) with either isotonic saline (IS, 0.15 M) or hypertonic saline (HS, 1.5 M) 30 or 90 min before decapitation.

**Experiment 2:** effects of acute hypovolemic stimuli on AVP release and gene transcription under euhydrated and dehydrated conditions. Rats in both euhydrated and dehydrated groups were injected ip (2% body wt) with either IS (0.15 M) or polyethylene glycol (PEG, MW 3000; Wako Pure Chemical Industries, Osaka, Japan) dissolved in IS (20% wt/vol) 90 min before decapitation.

**Measurements of plasma AVP, Na, and total protein.** After decapitation, trunk blood was collected into chilled tubes containing EDTA (potassium salt). Plasma 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 Kagaku Iatron, 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 (12). Plasma Na and total protein (TP) were measured with an autoanalyzer (Hitachi, Tokyo, Japan). The plasma TP levels were used to estimate acute changes in plasma volume (12, 17).

**Measurements of blood pressure.** To see whether ip injection of IS, HS, or PEG affected blood pressure, rats were placed in prewarmed chambers (35–36°C), and blood pressure was measured by an automatic tail cuff inflator and a built-in transducer with a photoelectric system (Promega, Madison, WI), 15 U of RNasin, 1 μl of 10 mM Tris (PerkinElmer Life Sciences, Natick, MA), the Riboprobe Combination System (Promega, Madison, WI), 15 U of RNasin, 1 μg of 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. Twelve-micrometer sections were cut on 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 × 10^6 counts/min of 33S-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 1X Denhardt’s solution). At the end of incubation, sections were subjected to consecutive washes in 4× standard saline citrate (SSC) for 15 min at room temperature and 50% formamide-250 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 to cool at room temperature and with 70% ethanol for 15 s. For analysis of AVP hnRNA, sections from each experimental group were placed in the same X-ray cassettes and exposed to Kodak BioMax MR films (Kodak, Rochester, NY) for 24–48 h. Changes in AVP hnRNA levels were examined by measurements of the integrated optical density (OD × area) of the film images, which were quantified using a computer image analysis system (Hamamatsu Photonics, Hamamatsu, Japan) and the public domain National Institutes of Health Image program. The mean values of AVP hnRNA expression levels in euhydrated rats injected with isotonic saline were expressed as 100.

Slides containing PVN were dipped in nuclear Kodak NTB2 emulsion (Kodak) and exposed for 3 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 levels of AVP expression, and their small dense-staining nuclei (5, 11).

**Statistics.** Statistical significance of the differences between groups was calculated by one-way ANOVA followed by Fisher’s protected least significant difference test. Results are expressed as means ± SE, and differences were considered significant at P < 0.05. The number of rats in each group was seven.

## RESULTS

**Experiment 1:** effects of acute osmotic stimuli on AVP release and gene transcription under euhydrated and dehydrated conditions. The AVP hnRNA expression levels in the SON and PVN, as well as the levels of plasma AVP, Na, and TP at 30 min after ip injection, are shown in Fig. 1 and Table 1. The ip injection of HS induced ~37 meq/l increases in plasma Na levels compared with ip injection of IS in euhydrated conditions (Table 1). AVP hnRNA expression levels in the SON and PVN, as well as plasma AVP levels, were significantly increased by HS injection compared with IS injection in euhydrated conditions (Fig. 1). On the other hand, although the ip injection of HS induced ~40 meq/l increases in plasma Na levels compared with IS injection in dehydrated conditions (Table 1), the AVP hnRNA levels in the SON and PVN were not significantly increased (Figs. 1, A and B, and 2). Although plasma AVP levels were significantly increased by HS injection in dehydrated conditions, the absolute values were significantly lower compared with those in euhydrated conditions (Fig. 1 C).

The plasma Na levels were still elevated at 90 min after HS injection in both euhydrated (IS, 139.0 ± 0.5 meq/l; HS, 168.1 ± 1.6 meq/l) and dehydrated conditions (IS, 147.2 ± 0.9 meq/l; HS, 188.6 ± 1.4 meq/l). The plasma AVP levels were also significantly elevated at 90 min after HS injection in both euhydrated (IS, 1.3 ± 0.2 pg/ml; HS, 62.7 ± 8.9 pg/ml) and dehydrated conditions (IS, 12.1 ± 1.3 pg/ml; HS, 27.6 ± 6.9 pg/ml). Although AVP hnRNA levels after HS injection were significantly increased in dehydrated conditions at 90 min in SON (IS, 100.0 ± 14.4; HS, 170.1 ± 9.5) and PVN (IS, 100.0 ± 16.7; HS, 199.1 ± 20.2), the levels were not significantly different between the IS and HS groups in dehydrated conditions (data not shown). The mean blood pressure in dehydrated conditions (109.8 ± 2.4 mmHg) was significantly (P < 0.01) higher than that in euhydrated conditions (86.9 ± 1.7 mmHg). The ip injection of HS, but not of IS, significantly increased the blood pressure in both euhydrated and dehydrated conditions (Table 2).

**Experiment 2:** effects of acute hypovolemic stimuli on AVP release and gene transcription under euhydrated and dehydrated conditions. In euhydrated conditions, plasma TP levels were increased after ip injection of PEG compared with IS
injection (IS, 5.6 ± 0.1 g/dl; PEG, 6.7 ± 0.1 g/dl; Table 3), by which plasma volume was estimated to have decreased by 19.7%. As reported previously (9), AVP hnRNA levels in the SON and PVN, as well as plasma AVP levels, were significantly increased by PEG injection compared with IS injection in euhydrated conditions (Fig. 3). In dehydrated conditions, plasma TP levels were increased after PEG injection compared with IS injection (IS, 6.3 ± 0.2 g/dl; PEG, 7.3 ± 0.2 g/dl; Table 3), by which plasma volume was estimated to have decreased by 16.1%. In contrast to HS injection, AVP hnRNA levels in the SON and PVN were significantly increased by PEG injection (Figs. 2 and 3, A and B). Plasma AVP levels were also increased significantly by PEG injection compared with IS injection in dehydrated conditions, and the absolute values were significantly higher than those in rats injected with PEG in euhydrated conditions (Fig. 3C). The arterial blood pressure did not change significantly at any time examined after ip injection of PEG in both euhydrated and dehydrated conditions (Table 2).

Microscopic analyses of emulsion-dipped slides revealed that, although AVP hnRNA was expressed robustly in the magnocellular neurons, virtually no signals in the parvocellular neurons were recognized in the PVN in any conditions examined (data not shown), indicating that changes in AVP hnRNA levels in the PVN reflected mainly changes in the magnocellular neurons.

**DISCUSSION**

In the present study, we have furthered our previous findings by showing that AVP gene transcription in the SON and PVN was completely refractory even to huge (~40 meq/l) increases in plasma Na levels, whereas AVP gene transcription increased in response to ~16% decreases in plasma volume in the rats that had been deprived of water for 3 days. Furthermore, our
Table 2. Changes in mean blood pressure in response to osmotic or hypovolemic stimuli under euhydrated and dehydrated (3 days water deprivation) conditions

<table>
<thead>
<tr>
<th>Time</th>
<th>Course, min</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euhydrated</td>
<td>IS</td>
<td>88.1±0.4</td>
<td>90.0±2.4</td>
<td>91.0±5.7</td>
<td>86.7±8.7</td>
<td>90.8±5.5</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>84.7±4.6</td>
<td>114.8±2.3*</td>
<td>115.1±4.0*</td>
<td>103.0±8.4*</td>
<td>95.3±7.7</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>87.9±2.4</td>
<td>90.1±9.1</td>
<td>90.0±4.1</td>
<td>85.8±2.8</td>
<td>90.5±0.8</td>
</tr>
<tr>
<td>Dehydrated</td>
<td>IS</td>
<td>109.1±2.5</td>
<td>109.4±4.2</td>
<td>108.6±2.1</td>
<td>100.7±6.2</td>
<td>103.1±4.6</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>107.1±5.0</td>
<td>126.0±5.7*</td>
<td>120.2±6.2*</td>
<td>117.7±3.7*</td>
<td>103.9±5.2</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>113.1±4.7</td>
<td>118.7±4.6</td>
<td>114.9±4.9</td>
<td>109.3±3.5</td>
<td>106.1±4.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. PEG, polyethylene glycol. Mean blood pressure (mmHg) was significantly elevated by ip injection (2% body wt) of 1.5 M HS, although it was not affected significantly by ip injection of PEG or IS. *P < 0.05 vs. values at time 0.

data showed that the response of AVP release to acute osmotic stimuli was blunted, whereas the response to hypovolemia was enhanced in the dehydrated rats.

We previously showed that AVP hnRNA expression in the SON and PVN was increased significantly as early as at 10 min, reached the maximum levels at 30 min, and started to decrease at 60 min after intraperitoneal injection of HS (0.45 M) in euhydrated rats (1). On the other hand, PEG is known to gradually decrease plasma volume (4), and the changes in plasma volume exceeded the threshold for AVP gene transcription as well as release at 90 min after intraperitoneal injection (9). The present study demonstrated that, under chronic dehydration, intraperitoneal injection of HS did not increase AVP hnRNA expression at 30 min, when the expression levels were expected to show the maximum, or at 90 min, when intraperitoneal injection of PEG increased AVP hnRNA expression in both euhydrated and dehydrated conditions, showing marked contrast between osmoregulation and volume regulation of AVP gene transcription.

Water deprivation not only decreases plasma volume but also increases plasma osmolality, and the rats had therefore been subjected to continued osmotic stimuli until the experiments in the present study. As pituitary AVP content decreases to ~50% levels after 3-day water deprivation (9), it is possible that the pathways to stimulate AVP synthesis in response to increases in plasma osmolality were fully activated under the chronic dehydration in efforts to compensate for the decreased pituitary AVP content. This might be the case not only for the signaling pathways within the AVP cells but also for the neural pathways involved in the osmoregulation such as organum vasculosum of the lamina terminalis. However, our data demonstrated that, although acute osmotic stimuli had no effect on driving AVP gene transcription in the SON and PVN, they still significantly increased AVP release after water deprivation. These data suggest that, although signals for increases in plasma osmolality were transferred to the magnocellular AVP neurons through neural inputs, the fully activated intracellular signaling pathways within the AVP cells made the gene refractory to increases in plasma osmolality. Furthermore, it is suggested that chronic dehydration and acute osmotic stimuli share the intracellular signaling pathways for AVP gene transcription.

Table 3. Changes in plasma Na and TP levels in response to hypovolemic stimuli under euhydrated and dehydrated (3 days water deprivation) conditions

<table>
<thead>
<tr>
<th></th>
<th>Euhydrated</th>
<th>Dehydrated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
<td>PEG</td>
</tr>
<tr>
<td>Plasma TP, g/dl</td>
<td>5.6±0.1</td>
<td>6.7±0.1*</td>
</tr>
<tr>
<td>Plasma Na, meq/l</td>
<td>138.1±1.0</td>
<td>137.5±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma TP levels were significantly increased 90 min after ip injection of 20% PEG compared with IS injection. *P < 0.05 vs. each IS group.
We previously measured the plasma volume of rats directly with Evans blue and reported that water deprivation for 3 days decreased plasma volume by ∼20% (9). The intraperitoneal injection of PEG further decreased (16.1%) the plasma volume of the dehydrated rats without affecting blood pressure and significantly increased AVP hnRNA expression in the present study, demonstrating that the signals of acute hypovolemia were conveyed to and upregulated AVP genes under chronic dehydration. Given that AVP gene transcription in the magnocellular neurons reaches maximal levels in terms of osmoregulation under chronic dehydration, these data suggest that AVP gene transcription could respond to acute hypovolemia probably through different intracellular signal transduction pathways from those for osmoregulation.

The response of AVP release to acute osmotic stimuli is enhanced if plasma volume is acutely reduced (4, 9). However, there are no such synergic effects under chronic dehydration, and these data have been interpreted as the resetting of volume regulation (7, 9, 13). In the present study, we have used a high amount of NaCl for injection and uncovered that the response of AVP release to acute osmotic stimuli was significantly attenuated under dehydrated conditions compared with euhydrated conditions. Although the attenuation might have been due to decreases in releasable AVP in pituitary stock, this would be unlikely because the response to hypovolemia was enhanced under dehydrated conditions. Although further study is warranted to elucidate how chronic dehydration affected the osmoregulation of AVP release, our data clearly demonstrated that chronic dehydration did not enhance, but rather blunted the response of AVP release to huge increases in plasma osmolality.

As shown in Table 2, the intraperitoneal injection of HS caused slight and temporal increases in blood pressure, which could potentially inhibit AVP gene transcription. However, as increases in blood pressure were similar between euhydrated and dehydrated conditions, these changes were unlikely to be related to different responses of AVP gene transcription in both conditions. The intraperitoneal injection of HS is also known as painful stress, and it is reported that AVP hnRNA was induced in the parvocellular neurons in the PVN after HS injection (10). However, long exposure of emulsion-dipped slides was usually necessary to visualize the signals, and virtually no signals were expressed in the parvocellular neurons in the PVN after 3-day exposure of emulsion-dipped slides when signals in the magnocellular neurons were highly expressed in the present study. These data indicate that the expression levels of AVP hnRNA in the parvocellular neurons were much lower than in the magnocellular neurons and that changes in AVP hnRNA expression levels in the parvocellular neurons had little effect on the analysis in the present study.

In conclusion, our data showed that acute reduction in plasma volume, but not increases in plasma osmolality, significantly increased AVP gene transcription in the SON and PVN in rats that had been deprived of water for 3 days, suggesting that signaling pathways regulating AVP gene transcription in the magnocellular neurons are different between osmo- and volume regulation. Clarifying the signaling pathways for each regulation remains a task for future investigations.

REFERENCES