Analysis of the vasopressin system and water regulation in genetically polydipsic mice

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1First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466–8550; 2Nagoya Higashi Municipal Hospital, Nagoya 464–0071; 3Anjo Kosei Hospital, Anjo 446–8602; and 4First Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807–0804, Japan

Yambe, Yuko, Yasuko Watanabe-Tomita, Satoshi Kakiya, Hisashi Yokoi, Hiroshi Nagasaki, Hiroshi Arima, Takashi Murase, Hiromitsu Yuasa, Kunikazu Kondo, Hiroshi Yamashita, and Yutaka Oiso. Analysis of the vasopressin system and water regulation in genetically polydipsic mice. Am. J. Physiol. Endocrinol. Metab. 278: E189–E194, 2000.—Polydipsic mice, STR/N, which show extreme polydipsia and polyuria, were discovered in 1958. In the STR/N, urine outputs are much higher than in control mice. The possibility of an abnormal regulation of the arginine vasopressin (AVP) system, or an abnormality in the renal susceptibility to AVP, should be considered. In this study we investigated the AVP system and water regulation in STR/N. We sequenced the AVP and the AVP V2-receptor genes of the STR/N by direct sequencing. No mutation was found in either of them. AVP gene expression examined by in situ hybridization and plasma sodium in 8-wk-old STR/N was significantly lower than in control mice, whereas it was significantly higher at 20 wk. Renal sensitivity to injected AVP was attenuated in 20-wk-old STR/N. The suppression of AVP synthesis due to excessive water retention in 8-wk-old STR/N suggests that polydipsia may be the primary cause in this strain. The 20-wk-old STR/N became dehydrated with the acceleration of AVP synthesis, which might have resulted from secondary desensitization to AVP.

polyuria; in situ hybridization; vasopressin V2-receptor gene

INBRED POLYDIPSIC MICE, STR/N, which show extreme polydipsia and polyuria with a recessive genetic trait, were discovered in 1958 by Silverstein and associates (17, 18). Daily water intake by the polydipsic STR/N mice was six times greater than by the control mice, and their urine output was also much greater. Many studies have been carried out thus far to clarify the mechanisms of drinking behavior and polyuria (3, 4, 8, 9, 13, 19).

In early studies, polydipsic mice showed high mortality only in males because of hydronephrosis caused by the combined presence of polyuria and urethral plugs at 6–12 mo of age. In the STR/N, neurosecretory granules were present in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) in the hypothalamus, and the STR/N could easily survive even if water intake were restricted (19). However, the details of the arginine vasopressin (AVP) system in STR/N have not been clearly elucidated.

Recent studies have revealed the presence of some functional abnormality in the central angiotensin (4, 9) and opioid (3, 8, 13) systems in STR/N, causing an increased thirst. Moreover, the number of AVP-containing neurons in the SON and PVN of polydipsic mice is significantly higher than in control mice (6), in contrast to AVP neurons in Brattleboro rats (21), which also have the symptoms of excessive thirst and increased urine output because of the lack of AVP by the mutation in the AVP genes (16). It has been reported that the distribution of AVP neurons in the hypothalamus of STR/N differs from that of control mice (6, 10, 20).

Therefore, the possibility of an abnormal regulation of the AVP system, or an abnormal renal susceptibility to AVP, should be considered, including the possibility of the mutation of the AVP gene and the AVP V2-receptor (V2R) gene. We supposed there were some etiological differences between polyuria in younger and older mice of STR/N.

In this study, to elucidate the etiology of polyuria in STR/N, we first sequenced the AVP gene and V2R gene of STR/N by direct sequencing. Second, AVP synthesis and water metabolism were examined in 8- and 20-wk-old STR/N.

METHODS

Animals. Male inbred polydipsic mice, STR/N strain, and their control, male ICR strain (also known as Swiss-Webster), at 8 and 20 wk of age were used. They were housed with three animals per cage under controlled conditions (23.0 ± 0.5°C; lights on, 0900–2100) and were provided dry food and tap water ad libitum. All procedures were performed in accordance with the institutional guidelines for animal care at the Nagoya University School of Medicine.

Analysis of AVP gene and V2R gene of STR/N. Genomic DNA was extracted from the thymus of ICR and STR/N using a QiAamp Tissue Kit (Quiagen, Germany). The AVP gene and V2R gene were amplified from genomic DNA by polymerase chain reaction as previously described (14). The entire coding region of the AVP and V2R genes from ICR and STR/N was sequenced by direct sequencing. No mutation was found in either of them.

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directly sequenced using primers shown in Table 1, as previously described (7). Because the mouse V2R gene had not been reported, we designed primer sets according to the rat V2R gene (11).

Changes in urine output, water intake, and plasma sodium. Basal urine output and water intake of STR/N at 8 and 20 wk of age were measured once a day for 3 days with animals in metabolic cages. After decapitation, trunk blood was collected into chilled tubes containing EDTA (potassium salt) for plasma sodium. Plasma sodium was measured using an autoanalyzer (Hitachi, Japan).

In situ hybridization. In situ hybridization was performed as previously described (1). The levels of AVP mRNA in SON and PVN of STR/N and ICR were examined at 8 and 20 wk of age, respectively. Briefly, removed brains were immediately frozen on powdered dry ice, sliced at 12 µm with a cryostat, and thaw-mounted onto gelatin-coated slides. The probe used was 35S-3'-end-labeled oligonucleotides complementary to part of the exonic mRNA sequences coding for the last sixteen amino acids of mouse AVP (2, 5), and it was applied to each section (2 x 105 counts/min). After overnight hybridization at 37°C, the sections were washed in 1x standard sodium citrate at 55°C for 15 min four times and at room temperature for 30 min twice, then air dried, and exposed to autoradiography film (Hyperfilm MP; Amersham International, Bucks, UK) for 24 h. The resulting images were analyzed using a Macintosh program (NIH Image) by comparison with simultaneously exposed 14C standards (Amersham) (12). The data representing the hybridization signals were expressed as a percentage of the control mice data.

Water restriction. To determine urine concentration ability, we measured urine output and urine osmolality of STR/N and ICR after water restriction for 3 h. Water restriction was stopped after only 3 h because urine output of ICR mice was too little to measure.

Renal sensitivity to AVP. To examine renal sensitivity to AVP in STR/N, urine osmolality was measured 2 h after intraperitoneal injection of synthetic AVP (Pitressin, Parke-Davis; 40 ng/g body wt) in STR/N and ICR. Intraperitoneal injection of isotonic saline was done as a control.

Table 1. Sequence of primer used for PCR and direct sequencing

<table>
<thead>
<tr>
<th>Primers for AVP Gene</th>
<th>Sequence</th>
<th>Location*</th>
</tr>
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<tbody>
<tr>
<td>A 5'-GTAGAAGCCACAGTGGTCGC-3' nt 1391-1410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 5'-TAACATTTCCTCTCTCTTA-3' nt 1695-1676</td>
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</tr>
<tr>
<td>C 5'-GTGCACTAGTTCCACCACG-3' nt 2700-2719</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 5'-TCCACTCTCTGGTCTGATC-3' nt 3006-3047</td>
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</tr>
<tr>
<td>E 5'-TTGCTTCAGAGACTGGTG-3' nt 3167-3186</td>
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<td></td>
</tr>
<tr>
<td>F 5'-GAGTGAAGGTTCCACCAGT-3' nt 3480-3461</td>
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</tbody>
</table>

AVP, arginine vasopressin. * Nucleotide (nt) numbering is according to the report of Hara et al. (2).

<table>
<thead>
<tr>
<th>Primers for V2R Gene</th>
<th>Sequence</th>
<th>Location†</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 5'-GTCATCATGACGGAGAGA-3' nt 20-39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H 5'-GATCGGCGAGATCATGTA-3' nt 442-423</td>
<td></td>
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</tr>
<tr>
<td>I 5'-ACACTGTTGATTTGCG-3' nt 855-874</td>
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<td></td>
</tr>
<tr>
<td>J 5'-CCTATGATGACGGATG-3' nt 1211-1192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K 5'-GGTGGGATGAGCCGCTT-3' nt 401-420</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 5'-GCGCCACGCGGGCAGAGA-3' nt 920-901</td>
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</tbody>
</table>

V2R, V2 receptor. † Nucleotide (nt) numbering is according to the report of Lolait et al. (11).

osmolality was measured by an automatic analyzer (Advanced Instruments).

Statistics. Results were expressed as means ± SE. Comparisons between groups was performed by Student's t-test. Differences were considered statistically significant at P < 0.05.

RESULTS

Sequence of the STR/N AVP gene. The entire coding region of the AVP gene of the STR/N strain was identical to that of the ICR strain. There was no mutation in the AVP gene of the STR/N strain. The 1,858th nucleotide of mouse AVP gene of the B10.A strain, which was previously reported by Hara et al. (2), is replaced by thymine in the AVP gene of STR/N and ICR. Nucleotide numbering is according to the report of Sausville et al. (15). Despite replacement of the nucleotide, the encoded amino acid was serine, which is the same as that of the B10.A mouse AVP gene.

Changes in water intake, urine output, and plasma sodium. Water intake was more than that of the control mice in both 8-wk-old STR/N (STR/N, 0.58 ± 0.09 ml·g body wt−1·day−1; ICR, 0.27 ± 0.02 ml·g body wt−1·day−1; n = 4, P < 0.05) and 20-wk-old STR/N (STR/N, 1.07 ± 0.13 ml·g body wt−1·day−1; ICR, 0.20 ± 0.01 ml·g body wt−1·day−1; n = 4, P < 0.01). Urine output in 8- and 20-wk-old STR/N also significantly increased compared with the ICR, both at 8 wk of age (STR/N, 0.25 ± 0.05 ml·g body wt−1·day−1; ICR, 0.04 ± 0.01 ml·g body wt−1·day−1; n = 4, P < 0.01) and at 20 wk of age (STR/N, 0.42 ± 0.01 ml·g body wt−1·day−1; ICR, 0.05 ± 0.01 ml·g body wt−1·day−1; n = 4, P < 0.01; Fig. 3). Plasma sodium was significantly lower than that of control in 8-wk-old STR/N (STR/N, 119.5 ± 3.5 meq/l; ICR, 135.3 ± 3.7 meq/l; n = 4, P < 0.01), whereas it was higher than the control in 20-wk-old STR/N (STR/N, 156.7 ± 5.3 meq/l; ICR, 139.5 ± 0.5 meq/l; n = 4, P < 0.05; Fig. 4).

AVP gene expression in SON and PVN. AVP mRNA levels in SON in 8-wk-old STR/N were significantly
lower than those of the control in both SON (STR/N, 44.9 ± 0.1%; ICR, 100.0 ± 0.2%, n = 6, P < 0.05) and PVN (STR/N, 207.4 ± 64.2%; ICR, 100.0 ± 12.3%, n = 6, P < 0.05; Fig. 5). Effect of water restriction. During water restriction for 3 h, urine output was very low in both STR/N and ICR. Urine output in STR/N was significantly higher than that of ICR at both 8 wk of age (STR/N, 0.011 ± 0.003 ml/g BW/day, ICR, 0.001 ± 0.003 ml/g BW/day, n = 4, P < 0.05; Fig. 2).
0.001 ml/g body wt; ICR, 0.003 ± 0.003 ml/g body wt; n = 4, P < 0.05) and 20 wk of age (STR/N, 0.020 ± 0.003 ml/g body wt; ICR, 0.003 ± 0.003 ml/g body wt; n = 4, P < 0.01). Urine output of 20-wk-old STR/N was significantly higher than that of 8-wk-old STR/N (P < 0.01). Urine osmolality of STR/N after water restriction was significantly lower than that of ICR only at 20 wk of age (8 wk: STR/N, 1,003.3 ± 153.0 mosmol/kg; ICR, 1,040.0 ± 98.8 mosmol/kg; n = 3, P < 0.05; 20 wk: STR/N, 660.7 ± 61.9 mosmol/kg; ICR, 1,147.0 ± 134.9 mosmol/kg; n = 3, P < 0.05).

Renal sensitivity to AVP. Urine osmolality of 8-wk-old STR/N after intraperitoneal injection of AVP significantly increased compared with the corresponding control (AVP-injected STR/N, 680.7 ± 87.9 mosmol/kg; saline-injected control, 524.7 ± 84.8 mosmol/kg; n = 3, P < 0.05; Fig. 6).

**DISCUSSION**

STR/N mice have a genetic abnormality characterized by extreme polydipsia and polyuria. Polydipsia and polyuria might be due to 1) disturbances in humoral control of water regulation, 2) excessive water intake resulting from a disturbed thirst regulation, or 3) both of the above.

AVP plays a critical role as the most important hormone in the maintenance of water metabolism through V2R. We first examined whether any mutation was found in the STR/N AVP gene. The nucleotides of the AVP gene in the STR/N were identical to those of ICR. The mouse AVP gene had been reported only in the
B10.A mouse (2). The 1,858th nucleotide of the AVP gene of STR/N and ICR was different from that of the B10.A strain. This difference did not affect the deduced amino acid. Second, we examined the STR/N V2R gene. The sequence of the mouse V2R gene has not been reported previously. On the basis of the rat V2R gene, we sequenced the V2R gene of ICR, and then the V2R gene of STR/N, our primary interest. The V2R gene of STR/N was the same as that of ICR. Thus the cause of the polydipsia and polyuria in STR/N is at least not an abnormality of the AVP and V2R genes.

The possibility of an abnormal regulation of the AVP system in STR/N should be considered. Polyuria in the STR/N occurred at 8 wk of age and became progressively more severe toward 20 wk of age. Hyponatremia and decreased mRNA levels were found in 8-wk-old STR/N, whereas they were increased in 20-wk-old STR/N. These findings indicate that the 8-wk-old STR is in an overhydrated state, whereas the 20-wk-old STR/N is in the dehydrated state.

Overhydration in 8-wk-old STR/N supports the idea that the STR/N mice become primarily polydipsic due to excessive thirst. These findings agree with the report by Katafuchi and Koizumi suggesting abnormal thirst in STR/N. They and colleagues suggested involvement of the central angiotensin system (3, 4, 8) and opioid system (9, 13) in polydipsia of the STR/N. Intracerebroventricular injection of angiotensin II inhibitors mildly reduced polydipsia in STR/N (9). On the other hand, water intake of the polydipsic STR/N mice was greatly reduced by an intracerebroventricular injection of opioid antagonists, especially by the specific k-opioid antagonist (8). It is speculated that the brain of STR/N probably contains more opioid and that particular opioid acts to increase spontaneous drinking (13).

The results of water restriction indicate that the urine concentration ability of 20-wk-old STR/N is impaired. We subsequently administered AVP to examine the renal sensitivity to it. Although STR/N showed polyuria at 20 as well as 8 wk of age, deterioration of the renal sensitivity to AVP was found only in the 20-wk-old STR/N in the present study. It is known that male polydipsic mice show hydrenephrosis caused by the combined presence of polyuria and urethral plugs. The plugs impeded the movement of a large volume of urine through the urethral passage. Hydrenephrosis of the STR/N males was more severe and led to high mortality (19). It is possible to conclude that AVP desensitization due to hydrenephrosis occurs in the 20-wk-old STR/N and that, with progressive polyuria, hypertonic dehydration develops with the acceleration of AVP synthesis. The result may be taken to mean that the 20-wk-old STR/N suffers from nephrogenic diabetes insipidus due to hydrenephrosis. The STR/N mice survived well, even when water intake was mildly restricted for 15 mo (19). This would suggest that the STR/N mice have an ability to produce concentrated urine to some extent, because AVP sensitivity attenuation is not critical.

In conclusion, the remarkable polydipsia of STR/N is not due to any mutation in the AVP or the V2R genes themselves. Interestingly, there were clear etiological differences between the polyuria in STR/N at 8 wk and 20 wk. The suppression of AVP synthesis and overhydration in 8-wk-old STR/N indicate the possibility that polydipsia may be the primary cause in STR/N. The 20-wk-old STR/N showed dehydration with the acceleration of AVP synthesis because of the secondary deterioration of AVP sensitivity due to the hydrenephrosis.

This work was supported by a Grant-in-Aid for Scientific Research Program (A1) from the Ministry of Education, Science, Sports, and Culture, Japan (no. 07507004) and the Research for the Future Program: The Japan Society for the Promotion of Science (JSPS-RFTF97I00201).

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Received 12 April 1999; accepted in final form 8 October 1999.

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