Impaired Urinary Concentrating Ability in Genetically Polyuric Mice

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Key Words

Hereditary polyuric mice · Resistance to vasopressin · CAMP · Adenylate cyclase · Cortical collecting ducts

Abstract

Newly recognized strain of mice with hereditary polyuria (PUS mice) was characterized. Polyuria was inherited as a single autosomal-recessive trait. At 15 weeks, PUS mice excreted hypotonic (urine osmolality: PUS; 270.8 ± 15.5 vs. cont.; $3,228.6 \pm 163.6$ mosm/kg) polyuria (urine volume: PUS; 25.0 ± 1.5 vs. cont.; 1.1 ± 0.1 ml/day). In PUS mice, plasma osmolality was slightly elevated as well as urinary excretion of vasopressin (AVP). Although PUS mice could concentrate urine after 24 h water deprivation, urine osmolality remained low. Blunted response to continuous infusion of dDAVP, a synthetic V2 agonist, was also observed. These in vivo studies indicated renal resistance to AVP contributed to the polyuria in this strain of mice. Microanalysis of isolated tubular segments revealed that AVP-induced cAMP accumulation in cortical collecting ducts (CCD) of PUS mice was significantly lower (60%) with or without a phosphodiesterase inhibitor, IBMX. Vasopressin induced similar cAMP accumulation in medullary ascending limbs of Henle (MAL),

and medullary collecting ducts (MCD) between PUS and control mice. In CCDs, PUS mice had low basal adenylate cyclase (AdC) activity and responded less to AVP and forskolin stimulation than control mice. No difference in cyclic AMP phosphodiesterase activity was detected between control and PUS mice. These results indicate that impaired cAMP accumulation due to low AdC activity may be related to the impaired renal concentrating ability observed in this new strain of mice.

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Introduction

Mammalian nephron has a wide range of urinary concentrating ability under various conditions such as dehydration and overhydration to keep plasma osmolality stable. Collecting ducts of the mammalian nephron concentrate the urine by increasing their permeability to water under high osmolar gradient in the renal medulla [1, 2]. Arginine vasopressin (AVP) mostly regulates this increase and contributes to building up the cortico-papillary osmolar gradient [1, 3]. The antidiuretic action of AVP in collecting ducts and medullary thick ascending limbs of Henle is mediated by V2 receptor [4]. Binding to V2

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Fig. 1. Effects of age on water intake and urine volume of PUS and control mice. Open circle = water intake; closed circle = urine volume of PUS mice. Open square = water intake; closed square = urine volume of control mice. Values are expressed as mean $\pm$ SE (n = 10 for each group).

receptor activates the G protein G_s resulting in the activation of adenylate cyclase (AdC), the production of cAMP and the stimulation of protein kinase A. In collecting ducts, this cascade of events leads to an insertion of specific water channel AQP2 into the luminal membranes [5], a final step of antidiuresis. Abnormality at any steps of this cascade can easily induce the urinary concentrating defect. Clinically, also experimentally, concentrating defect is observed as polyuria and is diagnosed according to its polyuric mechanisms, i.e. (1) central diabetes insipidus (DI), which lacks circulating bioactive AVP; (2) nephrogenic diabetes insipidus (NDI), in which condition the nephron shows unresponsiveness to AVP; (3) primary polydipsia (PP), i.e. excess intake of water [6], and (4) solute diuresis such as diabetes mellitus, diuretics usage, and hypercalcemia.

Recent advances in molecular technology have defined the mechanism of urinary concentrating defect especially in NDI [7, 8]. Ninety percent of congenital NDI patients had mutations in V2 receptor gene [8]. Mutations of AQP2 gene were also confirmed in some NDI patients [9]. In hereditary NDI mice, abnormally high catabolism of cAMP by high phosphodiesterase (PDE) activities caused renal resistance to AVP [10, 11]. Acquired forms of NDI are more common [12–14] and another mechanisms were reported. Among them, impaired AdC activities were observed in lithium-induced NDI rats [15]. These accumulated results indicate the diversity of mechanism responsible for NDI.

Fig. 2. Water balance and body weight of PUS and control mice. Values are expressed as mean $\pm$ SE of 10 mice from each group. * Significantly higher than corresponding values of female ($p < 0.05$, t test).

In the present study, we report a new strain of mice with congenital polyuria which mechanism is most likely partial nephrogenic.

Materials and Methods

Animals

Polyuric (further abbreviated as PUS) mice were first observed in 1984 during the crossing experiments of a strain of NOD mice with spontaneous diabetes mellitus [20] from C57BL/Shi mice. In addition to polyuria, PUS mice had agouti hair color. PUS and normal C57/BL mice were inbred in Shionogi Aburahi Laboratories with ordinary rat chow and tap water ad libitum. Growth curve, water intake and urine volume were monitored from 3 to 15 weeks (fig. 1, 2), and 10- 15-week-old polyuric mice and age-matched C57/BL mice as controls were subjected to the following experiments. To minimize variations between animals and between individual runs,

each experiment was conducted simultaneously on samples from control and PUS mice.

Genetic Analysis. Crossing experiments were performed between PUS and C57/BL mice to determine the inheritance style. A χ^2 test was applied to the statistical analysis of the segregation rate.

In vivo Studies

Metabolic Studies. PUS and control mice were placed into metabolic cages singly. Urine was collected for 24 h under mineral oil to prevent evaporation and blood sampling was conducted by gravity drainage after decapitation. Biochemical analysis of urine and blood was performed by standard laboratory analyzer. Urine osmolality was measured by depressing the freezing point and plasma osmolality was calculated by the following equation. $1.86 \times [\text{Na}] + [\text{glc}]/18 + [\text{BUN}]/2.8$, where $[\text{Na}]$ = serum Na concentration in mEq/l, $[\text{glc}]$ = plasma glucose level in mg/dl, and $[\text{BUN}]$ = blood urea nitrogen level in mg/dl. Plasma AVP levels after ether inhalation and urinary AVP levels collected for 24 h were measured by RIA [16].

Water Restriction Test. PUS and control mice in metabolic cages were deprived of water while having free access to the rat chow for 24 h. Urine was collected every 24 h before and during dehydration, and urine osmolality and urine volume were monitored.

dDAVP Infusion Test. Synthetic V2 agonist, 1-deamino-8-D-arginine-vasopressin (dDAVP) was continuously infused through osmotic minipump (model 1003D, Alza Corp., Palo Alto, Calif., USA) implanted in the abdominal subcutaneous region at the suitable flow rates delivering indicated concentrations.

In vitro Studies

Analysis of AVP-Dependent cAMP Accumulation, Adenylate Cyclase Activity and Phosphodiesterase Activity in Isolated Tubules

Isolation of AVP-Responsive Segments. Medullary ascending limbs of Henle (MAL), cortical collecting ducts (CCD), and medullary collecting ducts (MCD) were isolated from PUS and control mice as previously reported [11, 17]. In brief, mice were lightly anesthetized with pentobarbital sodium (6 mg/100 g body weight i.p.). The abdominal aorta was cannulated retrogradely and both kidneys were perfused to blanching with 5–7 ml of collagenase medium (for composition, see below, under 'Solutions'). After the perfusion, both kidneys were quickly removed, and cortical and outer medullary tissues were sliced with a blade. The slices were then incubated with collagenase medium aerated with a mixture of 95% O₂ and 5% CO₂, at 35 °C for 30–35 min. After incubation, segments of MALs, CCDs, and MCDs were dissected under stereomicroscope. For assays of activities of phosphodiesterase and adenylate cyclase, tubules were permeabilized by freezing and thawing in 0.25 μ l of hypotonic solution (composition given below) and stored at –80 °C until assay.

Measurement of cAMP Content in MALs, CCDs and MCDs. Dissected tubules (3–5 mm in length) were incubated in 2.5 μ l of KRB solution with 1 μ M AVP for 20 min at 30 °C in the presence of 0.5 mM PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX). The cAMP content was measured by RIA [17]. Accumulated cAMP was expressed as fmol/mm/20 min.

Measurement of AdC Activities in CCD. Adenylate cyclase activities were measured as described previously [11]. Permeabilized tubules were incubated with or without AVP in the presence of [α -³²P]ATP for 30 min at 30 °C in 2.5 μ l of incubation buffer. The incubation buffer contained (in mM): 0.25 [α -³²P]ATP (2–4 $\times$ 10⁶ counts/min/sample), 1 cAMP, 3.8 MgCl₂, 0.25 ethylene diamine

tetraacetic acid (EDTA), 1 ethylene glycolbis(fl-aminoethyl ether)-N, N, N',N'-tetraacetic acid (EGTA), 100 Tris. HCl, 20 creatine phosphate, and 1 mg/ml creatine kinase, pH 7.4. The reaction was stopped by placing slides onto dry ice and adding 150 μ l of stop solution containing (in mM): 3.3 ATP, 5 cAMP, 50 Tris.HCl and [³H]cAMP (1 $\times$ 10⁴ counts/min/sample) to monitor the cAMP recovery. Generated cAMP was separated through a Dowex 50W-X8 and aluminum column and radioactivity was counted by a liquid scintillation counter (model 3500, Aloka Co., Tokyo Japan). Adenylate cyclase activities were expressed as generated cAMP femtomoles/mm tubular length/30min.

Measurement of Phosphodiesterase (PDE) activities in CCD. Activities of low-affinity cAMP-PDE were measured by the methods previously reported by us with minor modifications [11]. Permeabilized tubules as described above were transferred together with cover slips into 12 $\times$ 75 mm polypolypropylene tubes and incubated with 50 μ l of incubation mixture (composition see below) for 10 min at 30 °C. The incubation mixture contained (in mM): 10 MgSO₄, 1 EGTA, 50 Tris·HCl and 1 μ M [³H]cAMP (Mg-EGTA-Tris buffer). Nucleotides were eluted from nucleosides through QAE-Sephadex A-25 columns with 20 mM acetic formate and radioactivity was counted by a liquid scintillation counter. PDE activity was expressed as hydrolyzed cAMP fmol/mm/min. Hydrolyzed cAMP was always less than 20% of total cAMP amount in each sample tube.

Since protein content of CCDs was not significantly different between control and PUS mice (control: 0.081 $\pm$ 0.004, PUS: 0.089 $\pm$ 0.012, μ g/mm), cAMP contents, AdC activities and PDE activities were expressed per mm tubular length.

Solutions and Materials

The microdissection medium was a modified Hank's solution, as used in our previous papers [17, 22]; 137 NaCl, 5 KCl, 0.8 MgSO₄, 0.33 Na₂PO₄, 1.0 MgCl₂, 10 Tris·HCl, and 0.25 CaCl₂, pH 7.4. The composition of collagenase media was identical to that of the microdissection media except it contains 1.0 CaCl₂ and 0.1% w/v collagenase. The hypotonic solution contains 1.0 MgCl₂, 0.25 EDTA, 0.1% bovine serum albumin, and 1.0 Tris·HCl, pH 7.4. Modified Krebs-Ringer buffer includes 140 NaCl, 5.0 KCl, 1.2 MgSO₄, 0.8 CaCl₂, 10 Na-acetate, 10 glucose, 20 Tris·HCl and 2.0 NaH₂PO₄, pH 7.4.

The following materials were purchased from the companies listed: collagenase (type I), bovine serum albumin, cAMP, snake venom, IBMX, aluminum oxide and dDAVP were from Sigma Chemical, St. Louis, Mo., USA; [α -³²P] ATP (74 MBq/ml), [³H]cAMP (37 MBq/ml) were from New England Nuclear, Boston, Mass., USA; RIA kits for cAMP measurement were from Biomedical Technologies Inc., Stoughton, Mass., USA; AVP and FK were from Calbiochem, San Diego, Calif., USA; QAE-Sephadex A-25 was from Pharmacia LKB Biotechnology, Uppsala, Sweden; AG 50W-x8 resin was from Japan Bio-Rad, Tokyo, Japan. RIA kits for AVP were from Mitsubishi Petrochemical Co., Ltd., Tokyo, Japan. Other chemicals were purchase from standard suppliers.

Statistical Analysis

Values were compared with unpaired Student's t test unless otherwise indicated.

Table 1. Genetic analysis of polyuric mice

Generations	Sex	Number of observations	Normal observed/expected*	Polyuria observed/expected*	χ^2	p
(C57BL × PUS) F1	F	12	12/12	0/0		
	M	14	14/14	0/0		
F2	F	57	44/42.7	13/14.3	0.16	0.5 < p < 0.7
	M	50	42/37.5	8/12.5	2.16	0.1 < p < 0.2
F1 × PUS	F	57	25/28.5	32/28.5	0.86	0.3 < p < 0.5
	M	59	33/29.5	26/29.5	0.83	0.3 < p < 0.5
PUS × PUS	F	82	0/0	82/82		
	M	89	0/0	89/89		

* Calculated numbers for single autosomal-recessive trait.
PUS = Polyuric strain.

Table 2. Biochemical analysis of blood and urine from control and PUS mice

a Blood

	Hematocrit %	Urea nitrogen mg/dl	Creatinine mg/dl	Na mEq/l	K mEq/l	Plasma protein g/dl	Glucose mg/dl
Control (n = 20)	46.5 ± 0.9	26.0 ± 0.9	0.4 ± 0.01	141.1 ± 0.4	5.6 ± 0.1	4.7 ± 0.1	171.6 ± 3.9
PUS (n = 16)	58.8 ± 0.2	28.4 ± 1.2	0.5 ± 0.02	148.6 ± 0.7*	5.6 ± 0.2	5.1 ± 0.1*	139.8 ± 5.5*

b Urine

	Urine flow ml/day	Urea nitrogen mg/dl	Creatinine mg/dl	Na mEq/l	K mEq/l	Protein g/dl	Glucose mg/dl
Control (n = 20)	0.9 ± 0.1	4446.4 ± 190.1 (35.7 ± 2.0)	71.7 ± 3.0 (0.6 ± 0.1)	129.4 ± 6.9 (0.10 ± 0.01)	263.3 ± 10.2 (0.21 ± 0.01)	0.8 ± 0.1 (0.01 ± 0.00)	not detected
PUS (n = 16)	17.1 ± 1.8*	822 ± 9.13* (117.2 ± 7.7)*	9.6 ± 1.1* (1.3 ± 0.1)*	21.1 ± 4.0* (0.26 ± 0.01)*	40.4 ± 3.8* (0.59 ± 0.04)*	0.3 ± 0.1* (0.05 ± 0.02)*	not detected

Biochemical parameters of urine were measured on samples collected for 24 h.

Values were expressed as mean ± SE of indicated number of animals.

* Significantly different from values of control mice (p < 0.05, unpaired t test).

Daily amounts were indicated in parentheses.

Results

Description of Polyuria

Polydipsia and polyuria were already detected in PUS mice as early as the weaning period (3 weeks) and was enhanced accordingly with growth (fig. 1). At around 10

weeks, water intake and urine volume reached plateau levels (intake 32.3 ± 2.0 ml, n = 20, urine 25.0 ± 1.5 ml, n = 20) which were almost equal to their body weight (fig. 2). PUS and control mice grew similarly and males (31.7 ± 0.9 g, n = 10 at 15 weeks) were bigger than females (24.1 ± 0.4 g, n = 10 at 15 weeks).

Table 3. Osmolality and AVP levels of plasma and urine from control and PUS mice

A Osmolality, mosm/kg H₂O

	Control	PUS
Plasma	281 ± 0.6 (n = 16)	294 ± 1.2 (n = 20)*
Urine	3,228 ± 163.6 (n = 20)	271 ± 15.5 (n = 20)**

B AVP

Plasma, pg/ml	1,691 ± 466 (n = 8)	2,050 ± 920 (n = 8)
Urine, pg/mg creatinine	873.5 ± 183.5 (n = 5)	1,163.3 ± 148.4 (n = 5)

Plasma AVP levels were measured under the maximum stimulation with ether inhalation. Urine AVP levels were measured on the samples collected for 24 h. Values were mean ± SE of samples denoted in the parentheses.

* Significantly higher than levels of control mice ($p < 0.001$, unpaired t test).

** Significantly lower than levels of control mice ($p < 0.001$, unpaired t test).

Genetic Analysis

Table 1 shows the results of the crossing experiments. Observed number of polyuria was well matched to the number calculated for a single autosomal recessive trait.

Chemical Analysis of Urine and Blood

As shown in table 2, PUS mice had higher hematocrit, plasma protein and serum Na levels indicating hemoconcentration. Blood glucose level was slightly lowered but no glycosuria was detected in PUS mice. Calculated plasma osmolality was slightly but significantly elevated in PUS mice than in control (table 3). PUS mice excreted hypotonic urine (tables 2, 3) with no significant proteinuria (table 2). Histological analysis revealed no significant abnormalities in glomeruli of PUS mice (data not shown). Renal function of PUS mice was normal as indicated by plasma creatinine levels. Plasma AVP levels stimulated maximally with ether inhalation and urinary AVP levels collected for 24 h tended to be elevated in PUS mice, but the difference was not significant (table 3).

Effects of Water Restriction

Twenty-four-hour water deprivation reduced urine volume and increased urine osmolality significantly both in PUS and control mice. Urine volume after water restriction was still higher in PUS mice (PUS: $2.2 \pm$

Fig. 3. Effects of exogenous dDAVP on urine volume and urine osmolality of PUS mice. PUS mice were treated with dDAVP using osmotic minipumps at rates of 25–2,500 ng/kg BW/day. Values are mean of 3–4 mice. Delivery rates were closed circle: 25 ng/kg BW/day, open circle: 250 ng/kg BW/day, closed square: 2,500 ng/kg BW/day.

0.1 ml/day, $n = 7$, control: 0.7 ± 0.1 ml/day, $n = 5$, $p < 0.05$). Urine osmolality of PUS mice after water restriction was significantly lower than that of control mice (PUS: 1521.8 ± 138.5 mosm/kg, $n = 7$, control: $3,675.0 \pm 228.2$ mosm/kg, $n = 5$, $p < 0.05$). After water restriction, PUS and control mice lost their body weight by 15.9 and 9.5%, respectively. Urinary AVP increased similarly both in PUS and control mice (data not shown).

Renal Response to Exogenous AVP

A synthetic AVP agonist dDAVP was continuously injected subcutaneously at the rate of 25, 250 and 2,500 ng/kg BW/day. Earlier report showed that 50–70 ng/kg BW/day of dDAVP successfully increased urine osmolality in Brattleboro rats, an animal model of central DI [18] as early as the following day of minipump implantation [19, 20]. As shown in figure 3, PUS mice did not respond to dDAVP infusion at the lower rates. Only the highest dose of dDAVP increased urine osmolality on the first day of minipump implantation and urine osmolality remained at a relatively stable level around 900 mosm/kg.

Fig. 4. AVP-induced cAMP accumulation in CCDs, MCDs, and MALs of control and PUS mice. Tubular samples were incubated with $1 \mu\text{M}$ AVP in the presence or absence of 0.5 mM IBMX for 20 min at 30°C . Values are mean $\pm$ SE of samples indicated at the bottom of each panel. Shaded bar = Control; hatched bar = PUS mice.

Fig. 5. Adenylate cyclase (AdC) activities in response to increasing dose of AVP in CCDs of control and PUS mice. Cortical collecting ducts (CCD) were incubated with indicated concentrations of AVP for 30 min at 30°C . Numbers are mean of 12–18 samples from 4 experiments. * Significantly higher than corresponding basal values. † Significantly higher than lower than control mice.

In vitro Study

AVP-Dependent cAMP Accumulation

cAMP accumulation was measured in AVP-responsive nephron segments, namely MALs, CCDs, and MCDs. Basal level of the cAMP content was always below the detectable range, therefore we compared the stimulated cAMP levels between PUS and control mice. In CCDs, cAMP contents stimulated maximally by $1 \mu\text{M}$ AVP were lower in PUS mice in the presence or absence of IBMX, a non-specific PDE inhibitor (fig. 4a). Vasopressin-dependent cAMP contents in MALs, and MCDs of PUS mice were not different from those of control mice (fig. 4b, c).

AVP-Dependent AdC Activities

To further examine how cAMP accumulation in CCDs of PUS mice was impaired, we measured AdC activities in this segment. Activities measured in permeabilized CCDs with various concentrations of AVP were summarized in figure 5. Basal activities were higher in control than in PUS mice (control; 27.9 ± 1.1 , $n = 17$. PUS; 17.0 ± 1.1 , $n = 16$, fmol/mm/30 min.). AVP stimulated AdC activities in a dose-dependent way as low as 100 pM both in control and PUS mice. Adenylate cyclase activities in

PUS mice were always lower at any dose of AVP except 1 pM and PUS mice responded less to the maximal stimulation with 1 μ M AVP than control mice by 40%. In separate experiments, 0.1 mM forskolin-stimulated AdC activities were also lower in PUS mice (cont.; 589.9 ± 80.5 , $n = 6$, PUS; 153.0 ± 7.8 , $n = 5$, $p < 0.05$, t test).

PDE Activities

The rate of hydrolysis of 1 μ M cAMP as a substrate was denoted as cAMP-PDE activities. Activities were slightly higher in CCDs of PUS mice as compared to control mice, but differences were not significant (table 4).

Discussion

In vivo Study

This new strain of mice of the present study seems to have different mechanisms for its polyuric state from previously reported congenital polyuric models [18, 21–24]. Polyuria developed in this strain of mice is apparently an inherited state as indicated by genetic analysis. Results of plasma and urinary AVP measurement with RIA indicated that PUS mice released the similar or slightly higher level of AVP compared to control mice. Since the antibody for this RIA system had low cross-reaction with dDAVP (0.19%), 8-lysine vasopressin (0.04%), oxytocin ($<0.01\%$), and vasotocin ($<0.01\%$) [16], it is unlikely that non-AVP substances such as oxytocin were detected as AVP. Water deprivation test showed no impairment of AVP release in PUS mice. In heterozygous Brattleboro rats, known as a partial central DI model, urine volume and osmolality were slightly different from control Long-Evans rats in spite of 20–40% reduction of circulating plasma AVP [18, 24], and less AVP was released during water deprivation in human cases with partial central DI [25]. Therefore, findings of the urine volume, plasma AVP levels and urinary AVP response to dehydration in the present study argue against the possibility that PUS mice have central DI.

Primary polydipsia resulting from defective osmoregulation of thirst was also unlikely. In primary polydipsia, AVP release is inhibited because the excess intake of water results in diluted plasma and lowered plasma osmolality. On the other hand, the renal response to AVP is preserved; therefore, urine can be concentrated when AVP release is stimulated by dehydration or when exogenous AVP is infused. As for PUS mice, higher plasma osmolality (table 3) and poor-responsiveness to exogenous AVP (fig. 4) was observed. Urinary AVP excretion

was not inhibited in PUS mice (table 3). These findings are contrary to those of primary polydipsia in human [6, 25].

PUS mice survived 24-hour dehydration and they could reduce the urine volume significantly. These results from the water deprivation were not completely against the possibility of nephrogenic DI, since mammalian nephron can concentrate urine through non-AVP-mediated mechanism [26, 27]. However these findings may indicate that renal unresponsiveness to AVP was partial. PUS mice showed poor-responsiveness to exogenous dDAVP, a potent antidiuretic agonist. We chose the dDAVP doses based on the experiments in Brattleboro rats. Continuous infusion of dDAVP with the same mini-pump system increased urine osmolality as low as 50 ng/kg BW/day in these rats [19, 20]. Therefore the required dose of 2,500 ng/kg BW/day in PUS mice (fig. 4) would be the supramaximal dose, even if the species difference was considered. From these *in vivo* findings, renal unresponsiveness to AVP seems most likely responsible for polyuria and polydipsia of this strain of mice.

In vitro Study

To further examine the mechanisms of renal resistance to AVP in PUS mice, we measured the AVP-dependent cAMP accumulation in isolated nephron segments, because cAMP is a well-documented second messenger to elucidate the increase in water permeability of AVP actions. Among the AVP-responsive collecting ducts, impaired cAMP accumulation was detected only in CCDs of PUS mice (fig. 4). Lower cAMP accumulation was accompanied by lower basal and AVP-dependent AdC activities (fig. 5). Impaired FK-induced AdC activities suggest that primary defect lies in catalytic unit. Previously, we reported abnormally high catabolism of cAMP by elevated PDE induced diuresis in congenital NDI mice and the polyuric state was successfully treated with specific PDE inhibitors [28]. Contrary to congenital NDI mice, no significant differences in cAMP-PDE activities were detected between PUS and control mice. These analysis of major components of cAMP metabolism indicate that the impairment of AdC activity is a main factor for lowered cAMP accumulation in CCDs of PUS mice.

Some hormones and cytokines are known to modify the AVP action in the kidney. Hydroosmotic effect of AVP was antagonized by α 2-adrenergic agonists or atrial natriuretic peptide [29, 30]. Alfa-adrenergic agonists also inhibited AVP-induced cAMP contents in rat CCDs [31]. Cytokines like TNF- β , IL-1 β and IL-2 increased AdC activities at basal as well as AVP- and FK-induced levels

in LLC-PK1 cells [32]. Although we did not evaluate these hormones, the presence of such inhibitors or absence of stimulators may be responsible for the impaired cAMP accumulation in PUS mice.

Although AVP increases water permeability in principal cells all along the collecting ducts, impaired cAMP accumulation was only detected in CCDs of PUS mice. We do not have the data explaining this limited abnormality, yet several possibilities can be considered such as changes in cell composition of CCDs, or presence of inhibitors. To what degree the impaired cAMP accumulation only in CCDs contributes to the polyuria documented in PUS mice remains in question. However, effects of impaired response to AVP in total CCDs on water diuresis could be significant, since water reabsorption in CCDs with antidiuresis is much greater than in MCDs and IMCDs [2]. Furthermore, as suggested as 'running start hypothesis' [33], urine cannot be concentrated to hypertonic unless large amount of water is reabsorbed in connecting tubules and CCDs, even if medullary and papillary collecting ducts are intact.

Medullary hypertonicity is another key factor for urine concentration. Countercurrent multiplication mechanism functions under high cortico-papillary osmolar gradient maintained by NaCl and urea reabsorption. In MALs, a segment that AVP increases NaCl transport through cAMP, AVP induced similar increase in cAMP accumula-

tion both in control and PUS mice. In contrast to Brattleboro rats [34], hypotrophy of MALs was not apparent in PUS mice. These results indicated NaCl absorption might be well preserved in this segment. Since we did not measure the tissue osmolality, we could not rule out possibility that diminished cortico-papillary osmolar gradient was responsible for polyuria as reported in some strain of mice [35]. Yet water diuresis itself lowers papillary osmolality mainly due to decreased urea content even in the presence of AVP, the condition in which tubules were permeable to water [36].

In summary, new mouse model of congenital polyuria was presented in this article. Its mechanism seemed multifactorial, yet renal unresponsiveness to AVP seems most likely responsible for polyuria and polydipsia of this strain of mice. Also impaired cAMP accumulation due to lowered AdC activities in CCDs may be related to the renal resistance to AVP. These abnormalities were different from previously reported polyuric models and this strain of mice will be the useful animal model to define the cellular mechanism of urine concentration.

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