Am J Physiol Renal Physiol 308: F261–F266, 2015.
First published December 10, 2014; doi:10.1152/ajprenal.00345.2014.

Effects of hydration in rats and mice with polycystic kidney disease

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Submitted 19 June 2014; accepted in final form 25 November 2014

Hopp K, Wang X, Ye H, Irazabal MV, Harris PC, Torres VE. Effects of hydration in rats and mice with polycystic kidney disease. *Am J Physiol Renal Physiol* 308: F261–F266, 2015. First published December 10, 2014; doi:10.1152/ajprenal.00345.2014.—Vasopressin and V2 receptor signaling promote polycystic kidney disease (PKD) progression, raising the question whether suppression of vasopressin release through enhanced hydration can delay disease advancement. Enhanced hydration by adding 5% glucose to the drinking water has proven protective in a rat model orthologous to autosomal recessive PKD. We wanted to exclude a glucose effect and explore the influence of enhanced hydration in a mouse model orthologous to autosomal dominant PKD. PCK rats were assigned to normal water intake (NWI) or high water intake (HWI) groups achieved by feeding a hydrated agar diet (HWI-agar) or by adding 5% glucose to the drinking water (HWI-glucose), with the latter group used to recapitulate previously published results. Homozygous *Pkd1* R3277C (*Pkd1*$^{RC/RC}$) mice were assigned to NWI and HWI-agar groups. To evaluate the effectiveness of HWI, kidney weight and histomorphometry were assessed, and urine vasopressin, renal cAMP levels, and phosphodiesterase activities were measured. HWI-agar, like HWI-glucose, reduced urine vasopressin, renal cAMP levels, and PKD severity in PCK rats but not in *Pkd1*$^{RC/RC}$ mice. Compared with rat kidneys, mouse kidneys had higher phosphodiesterase activity and lower cAMP levels and were less sensitive to the cystogenic effect of 1-deamino-8-D-arginine vasopressin, as previously shown for *Pkd1*$^{RC/RC}$ mice and confirmed here in *Pkd2*$^{WS25/-}$ mice. We conclude that the effect of enhanced hydration in rat and mouse models of PKD differs. More powerful suppression of V2 receptor-mediated signaling than achievable by enhanced hydration alone may be necessary to affect the development of PKD in mouse models.

polycystic kidney disease; vasopressin; cAMP; cyclic nucleotide phosphodiesterase; hydration

POLYCYSTIC KIDNEY DISEASES (PKDs) are characterized by the development and growth of cysts arising from renal tubules and associated with enlargement of the kidneys and destruction of the renal parenchyma. Autosomal dominant PKD (ADPKD), the fourth leading cause of end-stage kidney disease in adults, is caused by mutations to either of two genes, *PKD1* or *PKD2*. Autosomal recessive PKD (ARPKD), an important cause of end-stage renal disease and mortality in infants and children, is caused by mutations to polycystic kidney and hepatic disease 1 (*PKHD1*) (9, 23).

A large body of evidence indicates that vasopressin and V2 receptor signaling promote the progression of PKD via cAMP and PKA (22). Administration of the V2 receptor agonist 1-deamino-8-D-arginine vasopressin (DDAVP) aggravates the disease in orthologous models of ARPKD and PKD1 (10, 28). Genetic elimination of circulating vasopressin markedly inhibits the development of PKD in PCK rats, an effect that was reversed by the administration of DDAVP (28). Treatment with selective V2 receptor antagonists (mozavaptan or tolvaptan) inhibits renal cyst development in cpk mice (8) and in orthologous models of ARPKD (7, 26), PKD1 (10, 14), PKD2 (24, 25), and juvenile nephronophthisis (2, 7). A phase 3 randomized, double-blind clinical trial has shown that tolvaptan administered over 3 yr slows kidney growth and renal function decline in patients with ADPKD (20).

The effects of V2 receptor agonists and antagonists on PKD beg the question of whether suppressing vasopressin release through enhanced hydration can also delay disease progression. Indeed, Nagao et al. (16) showed that enhanced hydration by adding 5% glucose to the drinking water increased urine output 3.5-fold and slowed the progression of PKD in the PCK rat. Recently, the demonstration that phlorizin-induced glycosuria and osmotic diuresis are protective in Han:SPRD cy/+ rats has raised the question of whether the protective effect of 5% glucose in PCK rats could be due to glycosuria and increased urine flow rather than due to the suppression of vasopressin release (29). In addition, at this stage, no hydration studies have been performed in a mouse model or an orthologous ADPKD model. Therefore, the purpose of the present study was to determine whether suppressing vasopressin release through enhanced hydration using a hydrated agar diet would be protective in PCK rats and *Pkd1*$^{RC/RC}$ mice.

MATERIALS AND METHODS

Animal models. PCK rats (30) and C57BL/6 *Pkd1*$^{RC/RC}$ (10, 11), *Pkd2*$^{WS25/WS25}$, and *Pkd2*$^{+/-}$ (33) mice were maintained in the Animal Facilities of the Department of Veterinary Medicine of the Mayo Clinic (Rochester, MN). *Pkd2*$^{+/-}$ and *Pkd2*$^{WS25/WS25}$ mice were crossed to generate double-heterozygous *Pkd2*$^{WS25/-}$ mice. The Institutional Animal Care and Utilization Committee approved all experimental protocols for the work described within this report.

Increased hydration protocols. PCK rats were randomly assigned at 4 wk of age to control normal water intake (NWI) or to one of two high water intake (HWI) groups. HWI was achieved by adding 5% glucose to the drinking water (HWI-glucose) or by feeding a hydrated agar diet (HWI-agar) containing 5 g of powdered food (5053 regrind LabDiet), 50 ml of water, and 0.5 g of agar (Sigma-Aldrich) per 100 g body wt. Rats in NWI or HWI-glucose groups received the same amounts of powdered food and agar without added water. All groups had ad libitum access to water in bottles. At 6, 8, and 10 wk of age, rats were placed in metabolic cages to measure urine volume. Rats were killed at 10 wk of age for blood and tissue harvest.

Pkd1$^{RC/RC}$ mice were randomly assigned at 4 wk of age to NWI or HWI-agar groups. Mice in the HWI-agar group were fed 5 g of powdered food (5053 regrind LabDiet) and 0.25 g of agar (Sigma-Aldrich) in 25 ml of water per mouse. NWI mice received the same amounts of powered food and agar. Both groups had ad libitum access to water. Throughout the trial period, bottled water intake was measured weekly, and mice were placed in metabolic cages every 4 wk to measure urine output. NWI mice drank 4.08 ml·mouse^{-1}·day^{-1}, whereas HWI mice drank only 0.75 ml·mouse^{-1}·day^{-1}, from the bottles. HWI animals ate >90% of the food-agar mixture daily, providing a ~5.7-fold increase in daily water intake compared with

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NWI mice. All animals were euthanized at 24 wk of age for blood and tissue harvest.

Administration of DDAVP. DDAVP or saline vehicle was administered subcutaneously via osmotic minipumps (Alzet 2004 and 2ML4 for rats and Alzet 1004 for mice, replaced every 3 wk) to PCK rats (10 ng·100 g^{-1}·h^{-1}) between 3 and 10 wk of age (28) and to *Pkd1*$^{RC/RC}$ and *Pkd2*$^{WS25/-}$ mice (30 ng·100 g^{-1}·h^{-1}) between 4 and 12 wk of age and between 4 and 16 wk of age, respectively (10).

Urine vasopressin measurement. Urine vasopressin was measured in PCK rats and *Pkd1*$^{RC/RC}$ mice using an arg8-vasopressin EIA kit (ADI900-017, Enzo Life Science) according to the manufacturer's protocol. Urine from HWI-glucose and HWI-agar groups were concentrated using Centrifugal Filter Units (Millipore) before measurements.

Tissue and blood harvest/analysis. At death, animals were weighed and anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg ip) for rats or euthanized by CO_2 exposure (mice). Blood was obtained by cardiac puncture for the determination of serum creatinine and blood urea nitrogen levels. The right kidney and part of the liver were placed into preweighed vials containing 10% formaldehyde in phosphate buffer (pH 7.4). These tissues were embedded in paraffin for histological experiments. The left kidney was immediately frozen in liquid nitrogen for the determination of cAMP levels and phosphodiesterase (PDE) activities.

Histomorphometric analysis. Transverse tissue sections (4 μm) of the kidney, including the cortex, medulla, and papilla, and of the liver were stained with hematoxylin-eosin and picrosirius red to measure cystic and fibrotic indexes, respectively. Image-analysis procedures were performed with Meta-Morph software (Universal Imaging, West Chester, PA). Digital images were acquired using a light microscope with a high-resolution Nikon Digital camera (Nikon DXM 1200). The observer interactively applied techniques of enhancement for a better definition of interested structures and to exclude fields too damaged to be analyzed. A colored threshold was applied at a level that separated cysts from noncystic tissue and picrosirius red-positive material from background to calculate indexes of renal cysts as percentages of total tissue. Histomorphometric analyses were performed blindly, without knowledge of group assignment.

Immunohistology. Immunostaining for proliferating cell nuclear antigen (PCNA) with a monoclonal IgG2a antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used to measure epithelial cell proliferation. Fields (×400) of the renal medulla and cortex were randomly selected, and 1,000 tubular epithelial cell nuclei/tissue section were counted. Proliferative indexes were calculated as percentages of cells positive for PCNA.

Western blot analysis. Total ERK1/2, phosphorylated ERK1/2, and PCNA were measured by Western blot analysis using anti-ERK1/2, anti-phosphorylated ERK1/2, and anti-PCNA antibodies (Santa Cruz Biotechnology).

PDE activities. Kidneys were homogenized in ice-cold homogenization buffer containing 50 mM Tris (pH 7.5), 0.25 M sucrose, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, and 1 mM DTT and supplemented with a protease inhibitor tablets (Roche). PDE activities were measured using 1 μM cAMP as a substrate in buffer containing 50 mM Tris (pH 7.5), 5 mM $MgCl_2$, 4 mM 2-mercaptoethanol, and 0.1% BSA. [^{3}H]cAMP was included as a tracer for quantitation. Assays were initiated by the addition of substrate and incubated for 10 min at 30°C. The reaction was stopped by incubation for 3 min in boiling water. *Crotalus atrox* snake venom was then added, and, after 15 min of incubation at 30°C, hydrolyzed nucleotides were separated using high-capacity preactivated ion exchange resin (FabGennix, Frisco, TX). Slurries were mixed thoroughly and left to stand for 15 min on ice before centrifugation at 12,000 *g* for 3 min. The radioactivity in 150-μl aliquots of the resulting supernatants was determined by liquid scintillation counting. To determine the activity in a sample due to a specific PDE, various activators or inhibitors were included in the assay. PDE activity in aliquots incubated without Ca^{2+} and with 2 mM EGTA was determined as basal activity. Calmodulin-stimulated PDE1 activity was determined by subtracting basal activity from the activity in the presence of 2.01 mM $CaCl_2$ and 10 μg/ml calmodulin. PDE3 and PDE4 activities were determined as cAMP-PDE inhibitable by 10 μM cilostamide or rolipram, respectively. Hydrolysis of cAMP was linearly proportional to incubation time and enzyme protein. Specific activities were defined as picomoles of cAMP hydrolyzed per minute per milligram of protein (27).

Renal cAMP content. The kidneys were ground to fine powder under liquid nitrogen in a stainless steel mortar and homogenized in 10 volumes of cold 5% TCA in a glass-Teflon tissue grinder. After centrifugation at 600 *g* for 10 min, supernatants were extracted with 3 volumes of water-saturated ether. After the aqueous extracts had been dried, reconstituted samples were processed without acetylation using an enzyme immunoassay kit (Sigma-Aldrich). Results are expressed as picomoles per milligram of protein.

Statistical analysis. Data are expressed as means ± SD. Comparisons between groups were performed by a *t*-test.

RESULTS

Effect of enhanced hydration in PCK rats. Enhanced hydration in PCK rats, achieved by adding 5% glucose to the drinking water (HWI-glucose, $n = 10$ male rats and 10 female rats) or by feeding a hydrated 1% agar diet (HWI-agar, $n = 10$ male rats and 10 female rats) between 4 and 10 wk of age, resulted in significant and similar fourfold increases in urine output compared with NWI rats ($n = 10$ male rats and 10 female rats). Both treatments were associated with significant overall reductions in urine vasopressin and renal levels of

Table 1. *Effect of enhanced hydration on the development of polycystic kidney disease in PCK rats*

	Male Animals			Female Animals		
	NWI	HWI-agar	HWI-glucose	NWI	HWI-agar	HWI-glucose
Body weight, g	398.7 ± 19.5	363.6 ± 16.7‡	392.7 ± 19.8	243.7 ± 17.9	228.8 ± 9.92*	246.1 ± 14.1
Total kidney weight, g	5.72 ± 0.84	3.63 ± 0.39‡	4.45 ± 0.68†	2.97 ± 0.21	2.31 ± 0.18‡	2.52 ± 0.22‡
Kidney weight, %body weight	1.44 ± 0.24	1.00 ± 0.12‡	1.13 ± 0.14†	1.22 ± 0.02	0.99 ± 0.07‡	1.02 ± 0.07‡
Kidney cystic index, %	21.9 ± 6.13	15.1 ± 6.56*	17.0 ± 3.74*	18.1 ± 3.75	14.3 ± 3.60*	14.4 ± 4.11*
Kidney fibrotic index, %	2.24 ± 1.62	0.81 ± 0.47*	0.83 ± 0.71*	2.14 ± 0.59	1.02 ± 0.43‡	1.08 ± 0.35‡
Renal cAMP, pmol/mg protein	16.3 ± 5.67	12.0 ± 2.33*	11.3 ± 5.26	16.4 ± 8.0	8.29 ± 2.20*	8.78 ± 2.92*
Plasma urea, mg/dl	50.7 ± 3.65	42.7 ± 2.35‡	41.9 ± 2.25‡	48.6 ± 4.48	41.4 ± 3.93†	38.0 ± 3.20‡
Plasma creatinine, mg/dl	0.36 ± 0.05	0.31 ± 0.04*	0.36 ± 0.02	0.39 ± 0.04	0.35 ± 0.03*	0.36 ± 0.02
24-h urine, ml	15.8 ± 4.08	62.6 ± 13.5‡	61.5 ± 27.2‡	9.20 ± 1.48	53.5 ± 3.5†	53.7 ± 17.0‡
Urine vasopressin, pg/24 h	830 ± 400	251 ± 61†	43 ± 47‡	607 ± 172	301 ± 67‡	81 ± 16‡
Blood pressure, mmHg	117.5 ± 6.77	119.5 ± 5.99	120.0 ± 5.77	119.5 ± 6.85	120.5 ± 7.25	119.7 ± 5.66

Values are means ± SD; $n = 10$ animals/group. NWI, normal water intake; HWI-agar, high water intake (HWI) with the addition of a hydrated agar diet; HWI-glucose, HWI with the addition of 5% glucose to the drinking water. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ compared to the NWI group.

cAMP, with a marked protective effect on the development of PKD, as reflected by lower kidney weights, cystic and fibrotic indexes, plasma urea, and (HWI-agar only) plasma creatinine (Table 1 and Fig. 1*A*). Furthermore, ERK phosphorylation and cell proliferation pathways stimulated by vasopressin were significantly downregulated in HWI rats (Fig. 2, *A*, *C*, and *E*). There was no detectable effect on tail cuff blood pressures (Table 1).

Effect of HWI-agar in Pkd1$^{RC/RC}$ mice. As in PCK rats, urine flow rates were markedly increased in HWI-agar (n = 12 male mice and 12 female mice) compared with NWI *Pkd1*$^{RC/RC}$ mice (n = 12 male mice and 12 female mice; Table 2). This, however, was not accompanied by significant effects on urine vasopressin, renal cAMP, ERK phosphorylation, cell proliferation, kidney weight, cystic and fibrotic indexes, and plasma urea or plasma creatinine levels (Table 2 and Figs. 1*B* and 2, *B*, *D*, and F).

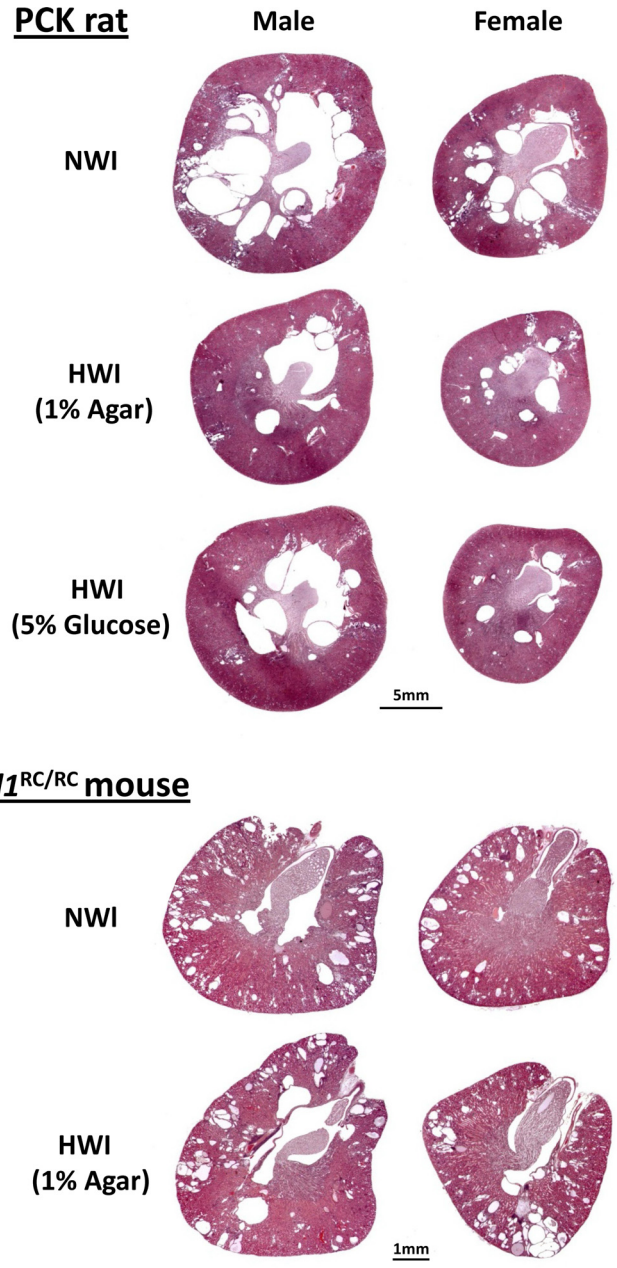

Fig. 1. *A*: representative hematoxylin and eosin-stained kidney cross-sections from PCK rats on normal water intake (NWI; drinking water ad libitum) or on enhanced hydration [high water intake (HWI)] induced by adding 5% glucose to the drinking water (5% glucose) or by administration of a hydrated agar diet (1% agar). *B*: representative hematoxylin and eosin-stained kidney sections from *Pkd1*$^{RC/RC}$ mice on NWI or on HWI (1% agar).

cAMP PDE activities and cAMP levels in PCK rats versus Pkd1$^{RC/RC}$ mice. In a previous study (27), we showed that renal PDE activities are higher in wild-type and PKD (*Pkd2*$^{WS25/-}$) mice compared with wild-type and PKD (PCK) rats (27). Hence, we wondered whether the inability to demonstrate a beneficial effect of enhanced hydration in *Pkd1*$^{RC/RC}$ mice could be due to increased PDE activity and lower basal levels of renal cAMP, which would require a more powerful suppression of V2 receptor-mediated signaling than that achievable by enhanced hydration alone. Indeed, renal PDE activities were significantly higher and cAMP levels were significantly lower in *Pkd1*$^{RC/RC}$ mice compared with PCK rats (Fig. 3).

Effects of DDAVP in Pkd1 and Pkd2 mouse models compared with PCK rats. If the different ability to demonstrate an effect of water hydration in *Pkd1*$^{RC/RC}$ mice compared with PCK rats was due to higher PDE activity, we reasoned that rat models of PKD would be more sensitive than mouse models to the cystogenic effects of the V2 receptor agonist DDAVP. In previous studies (10, 28) where we ascertained the effect of DDAVP on the development of PKD, this appeared to be larger in PCK rats than in *Pkd1*$^{RC/RC}$ mice. To confirm this observation in a different mouse model of PKD, we treated *Pkd2*$^{WS25/-}$ mice with DDAVP. The combined results show that the administration of DDAVP at 10 ng·100 g^{-1}·h^{-1} increased the kidney weight-to-body weight ratio by 256% and 301% in PCK rats, whereas DDAVP at 30 ng·100 g^{-1}·h^{-1} increased the kidney weight-to-body weight ratio by only 38% and 46% in *Pkd1*$^{RC/RC}$ mice and by 27% and 79% in *Pkd2*$^{WS25/-}$ mice (both male and female mice, respectively; Fig. 4).

DISCUSSION

A large body of evidence indicates that the vasopressin V2 receptor and cAMP signaling play an important role in the pathogenesis of PKD (22). Based on this evidence, a recommendation has been made that patients with ADPKD and normal renal function increase the amount of solute-free water drunk evenly throughout the day to decrease plasma vasopressin concentrations and mitigate the action of cAMP on renal cysts (19). Although the hypothesis that enhanced hydration may slow the progression of ADPKD has not been adequately tested in patients, Nagao et al. (16) showed that enhanced hydration induced by the addition of 5% glucose to the drinking water suppresses vasopressin/V2 receptor signaling, reduces the renal levels of cAMP, and inhibits the development of PKD in the PCK rat, a model orthologous to ARPKD. The possibility that the beneficial effect of adding 5% glucose to the drinking water was due to the induction of glycosuria and increased urine flow rather than to the suppression of vasopressin has been raised by a recent report (29) showing that the development of glycosuria and osmotic diuresis by phlorizin-induced inhibition of Na^+-glucose cotransporters ameliorated PKD in Han:SPRD Cy/+ rats. Our study has shown an equally protective effect when hydration was induced by the utilization

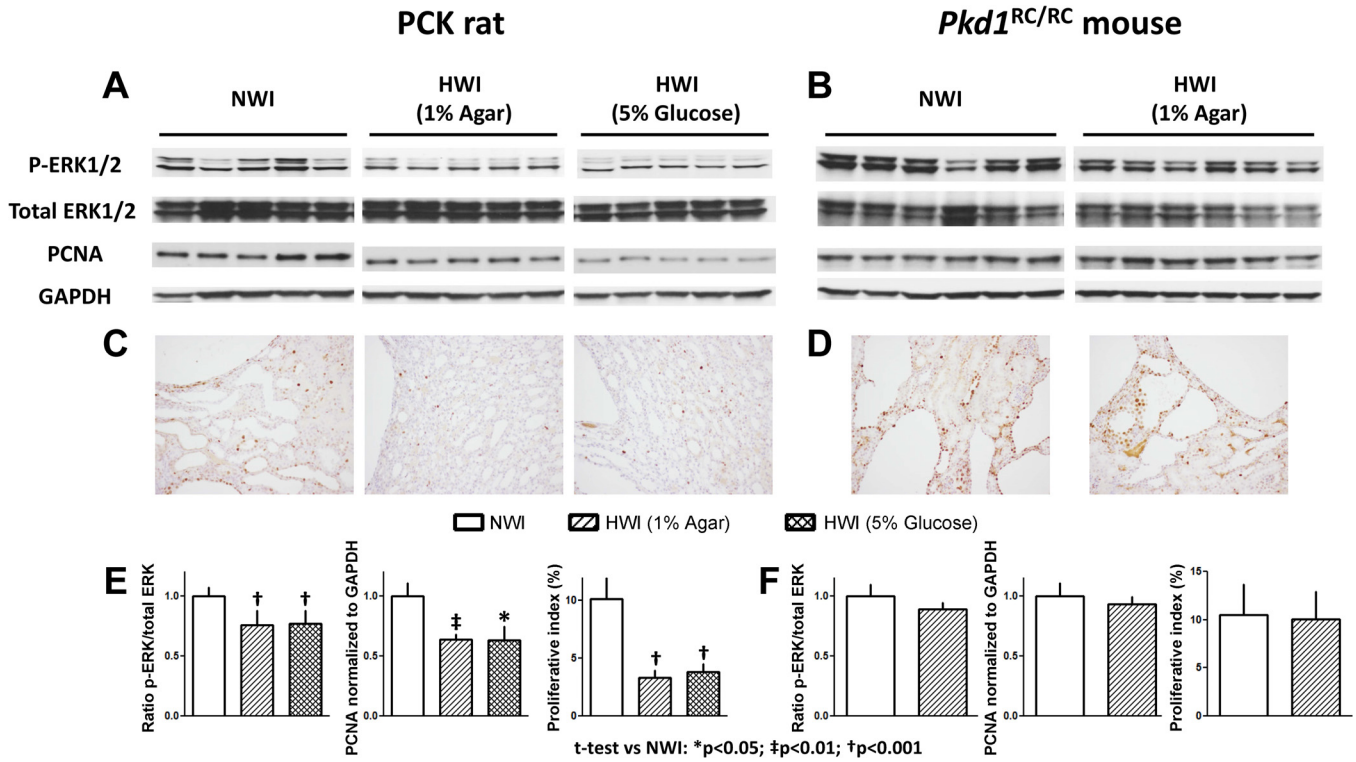

Fig. 2. Western blot analysis of total and phosphorylated (P) ERK1/2, proliferating cell nuclear antigen (PCNA), and GAPDH in whole kidney lysates. ERK1/2 phosphorylation and PCNA expression were significantly reduced in PCK HWI rats (*A* and *E*) but not in *Pkd1*$^{RC/RC}$ HWI mice compared with their NWI controls (*B* and *F*). For PCNA immunostaining, proliferative indexes were reduced in HWI PCK rats (*C* and *E*) but not in HWI *Pkd1*$^{RC/RC}$ mice (*D* and *F*) compared with NWI controls. $n = 10$ PCK rats/group in *E* and 12 *Pkd1*$^{RC/RC}$ mice/group in *F*.

of a hydrated agar diet. This was associated with reduced urine vasopressin excretion and renal levels of cAMP, making it unlikely that the beneficial effect of adding 5% glucose to the drinking water was due to the induction of glycosuria or due to metabolic effects of the glucose load.

In contrast to its protective effect in the PCK rat model, enhanced hydration did not slow PKD progression in *Pkd1*$^{RC/RC}$ mice, and urine vasopressin levels remained unchanged in HWI mice despite their ~10-fold greater urine output. This lack of urine vasopressin suppression may be consistent with a report (1) of inappropriate expression of vasopressin in the brain of *Pkd1*$^{+/-}$ mice despite chronic low plasma osmolality or due to higher urine concentrating abilities of mice compared with rats, which thus require greater water loading to reduce vasopressin levels (34). Furthermore, it should be considered that urine vasopressin levels do not reliably reflect generation or plasma levels of vasopressin (4, 17, 18). For example, diuresis from a large water load may be accompanied by a transient paradoxical rise in vasopressin excretion while plasma osmolality decreases (17). Therefore, we cannot definitely conclude from our results that vasopressin release was not suppressed in HWI *Pkd1*$^{RC/RC}$ mice and how this correlated with the lack of disease alleviation.

Additional factors might have contributed to these unexpected results. Since vasopressin V2 receptor antagonists are effective in PCK rats, *Pkd1*$^{RC/RC}$ mice (10), and *Pkd2*$^{WS25/-}$ mice (24, 25), it seems unlikely that the genes mutated (PCK rat: *Pkhd1*; *Pkd1*$^{RC/RC}$ mouse: *Pkd1*; and *Pkd2*$^{WS25/-}$ mouse:

Table 2. *Effect of enhanced hydration on the development of polycystic kidney disease in Pkd1*$^{RC/RC}$ *mice*

	Male Animals		Female Animals	
	NWI	HWI-agar	NWI	HWI-agar
Body weight, g	28.0 ± 2.6	29.1 ± 2.5	21.5 ± 0.9	23.0 ± 1.0†
Total kidney weight, g	0.58 ± 0.12	0.58 ± 0.09	0.43 ± 0.03	0.47 ± 0.11
Kidney weight, %body weight	2.05 ± 0.29	2.00 ± 0.18	2.02 ± 0.11	2.04 ± 0.40
Kidney cystic index, %	13.0 ± 4.5	15.66 ± 4.90	14.55 ± 2.62	16.79 ± 4.37
Kidney fibrotic index, %	1.50 ± 1.35	1.58 ± 1.15	2.07 ± 1.25	2.10 ± 1.38
Renal cAMP, pmol/mg protein	6.55 ± 3.73	7.07 ± 3.29	6.18 ± 1.40	8.15 ± 3.87
Plasma urea, mg/dl	46.8 ± 13.4	45.9 ± 6.46	43.7 ± 8.22	36.1 ± 7.2*
Plasma creatinine, mg/dl	0.22 ± 0.03	0.22 ± 0.04	0.20 ± 0.03	0.18 ± 0.03
24-h urine, ml	1.7 ± 0.4	13.7 ± 2.0‡	1.5 ± 0.6	10.0 ± 0.6†
Urine vasopressin, pg/12 h	172 ± 66	196 ± 113	75 ± 61	182 ± 71

Values are means ± SD; $n = 12$ animals/group. *$P < 0.05$ and †$P < 0.001$ compared with the NWI group.

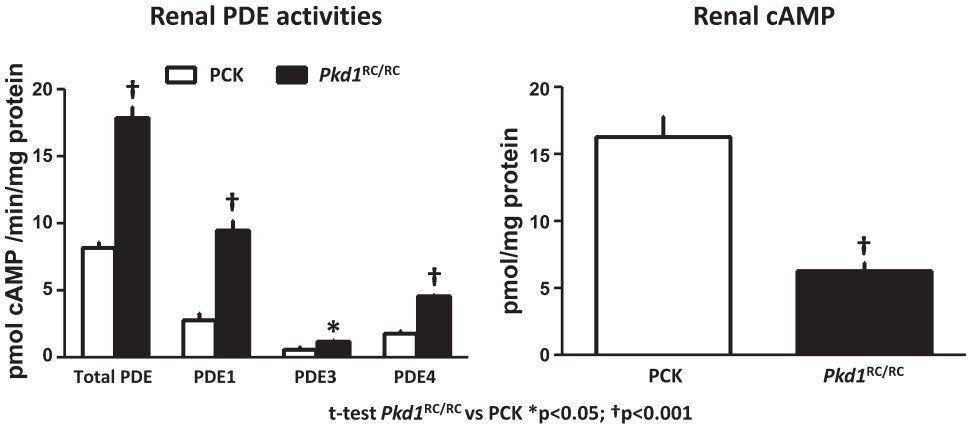

Fig. 3. Total phosphodiesterase (PDE) activites (PDE1, PDE3, and PDE4) and cAMP levels measured in whole kidney tissue homogenates from PCK rats and *Pkd1*$^{RC/RC}$ mice. For PDE activity, $n = 10$ animals/group and model; for cAMP levels, $n = 20$ PCK rats and 24 *Pkd1*$^{RC/RC}$ mice.

Pkd2) account for the different effects of hydration in these animal models.

Instead, differences in intrinsic susceptibility to the development of PKD between rats and mice may be a contributory factor. In this regard, it is telling that the PCK rat exhibits renal cysts at or soon after birth (13), whereas most *Pkhd1* knockout mice only develop renal cysts at an advanced age or not at all (3, 5, 6, 15, 31, 32). The renal phenotype of *Pkd1* or *Pkd2* heterozygous knockout mice is similarly normal or very mild; unfortunately, no *Pkd1* or *Pkd2* rat model currently exists (21). Furthermore, in previous studies, we and others (12, 27) reported higher PDE activities in kidneys from mice compared with those from rats. Since the hydrolytic capacity of PDEs far exceeds the maximum rate of synthesis by adenylyl cyclases, cellular levels of cAMP may be more sensitive to changes in PDEs compared with those in adenylyl cyclases. Therefore, a variation in cyclic nucleotide PDE activities may determine variable susceptibility to renal cystic disease. The observations in the present study, namely, the lower renal cAMP PDE activities and higher cAMP levels, as well as the increased susceptibility to the cystogenic effect of DDAVP in rats compared with mice, provide support for this hypothesis.

ACKNOWLEDGMENTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-44863 (to V. E. Torres), DK-058816 (to P. C. Harris), and DK-090728 (Mayo Translational PKD Center), the ASN Ben J. Lipps Fellowship (to K. Hopp), and the Zell Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.H., X.W., P.C.H., and V.E.T. conception and design of research; K.H., X.W., and H.Y. performed experiments; K.H., X.W., and V.E.T. analyzed data; K.H., X.W., M.V.I., and V.E.T. interpreted results of experiments; K.H., X.W., and V.E.T. prepared figures; K.H., X.W., and V.E.T. drafted manuscript; K.H., X.W., H.Y., M.V.I., P.C.H., and V.E.T. edited and revised manuscript; K.H., X.W., H.Y., M.V.I., P.C.H., and V.E.T. approved final version of manuscript.

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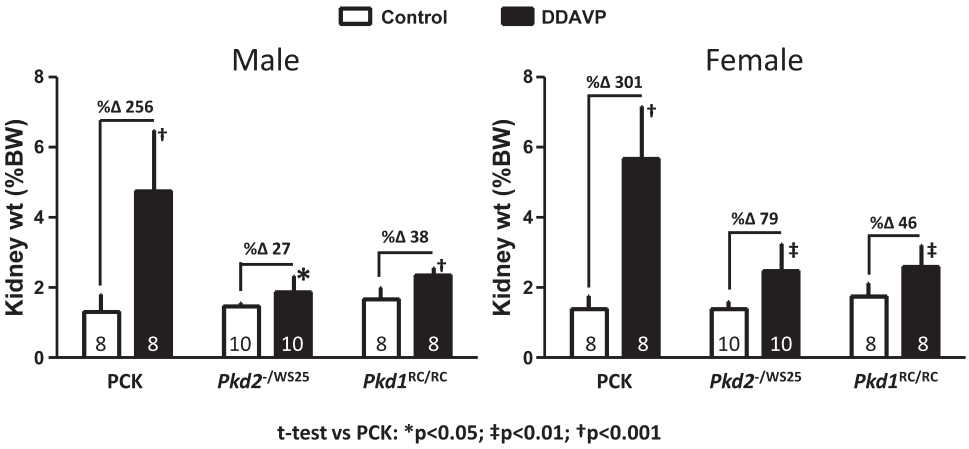

Fig. 4. Effect of vehicle or 1-deamino-8-D-arginine vasopressin (DDAVP) administration to PCK rats, *Pkd1*$^{RC/RC}$ mice, and *Pkd2*$^{WS25/-}$ mice on disease severity (reflected by percent kidney weight/body weight). The data on PCK rats and *Pkd1*$^{RC/RC}$ mice have been previously published (10, 28).

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