

Vasopressin Directly Regulates Cyst Growth in Polycystic Kidney Disease

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ABSTRACT

The polycystic kidney diseases (PKD) are a group of genetic disorders causing renal failure and death from infancy to adulthood. Arginine vasopressin (AVP) V2 receptor antagonists inhibit cystogenesis in animal models of cystic kidney diseases, presumably by downregulating cAMP signaling, cell proliferation, and chloride-driven fluid secretion. For confirmation that the protective effect of these drugs is due to antagonism of AVP, PCK (*Pkhd1*^{-/-}) and Brattleboro (*AVP*^{-/-}) rats were crossed to generate rats with PKD and varying amounts of AVP. At 10 and 20 weeks of age, PCK *AVP*^{-/-} rats had lower renal cAMP and almost complete inhibition of cystogenesis compared with PCK *AVP*^{+/+} and PCK *AVP*^{+/-} rats. The V2 receptor agonist 1-deamino-8-D-arginine vasopressin increased renal cAMP and recovered the full cystic phenotype of PCK *AVP*^{-/-} rats and aggravated the cystic disease of PCK *AVP*^{+/+} rats but did not induce cystic changes in wild-type rats. These observations indicate that AVP is a powerful modulator of cystogenesis and provide further support for clinical trials of V2 receptor antagonists in PKD.

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common of the inherited renal cystic diseases and a leading cause of ESRD. It is genetically heterogeneous with two genes identified, *PKD1* and *PKD2*.¹ Autosomal recessive PKD is less common than ADPKD but together with nephronophthisis is a leading cause of ESRD in childhood. It is caused by mutations to *PKHD1*. The proteins encoded by *PKD1* (polycystin-1), *PKD2* (polycystin-2), and *PKHD1* (fibrocystin/polyductin) are membrane-associated proteins. The downstream signaling pathways associated with these proteins provide potential therapeutic targets.² Arginine vasopressin (AVP) V2 receptor antagonists (OPC31260 and OPC41061) inhibit cystogenesis in animal models orthologous to human autosomal recessive PKD (PCK rat), ADPKD (*Pkd2*^{-/tm1Som}), and adolescent nephronophthisis (pcy mouse) by downregulating cAMP signaling, cell proliferation, and chloride-driven fluid secretion.^{3–5} Clinical trials with V2 receptor antagonists have been initiated. The mechanism by which these drugs inhibit the development of polycystic kidney disease has not been fully elucidated. In addition to

V2 antagonism, these drugs could exert an effect by favoring the action of AVP on V1a receptors or by an AVP unrelated mechanism.

RESULTS AND DISCUSSION

To confirm that inhibition of AVP action on the kidney accounts for the effect of V2 receptor antagonists on the development of PKD, we generated PCK *AVP*^{+/+}, PCK *AVP*^{+/-}, and PCK *AVP*^{-/-} rats, as well as wild-type and Brattleboro controls, by breeding F1 rats resulting from PCK (*Pkhd1*^{-/-}) and Brattleboro (*AVP*^{-/-}) crosses. PCK rats are homozygous for a splicing mutation (IVS35-2A→T)

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that skips exon 36 and leads to a frame shift in *Pkhd1*.⁶ Brattleboro rats lack circulating AVP and are homozygous for a 1-bp deletion of a guanine nucleotide in the second exon of the *AVP* gene that results in a frame shift of the coding sequence for the carrier neurophysin II.⁷ Approximately 10 male and 10 female

rats of each genotype were killed at 10 and 20 wk of age (Figures 1 and 2, A and B; Supplementary Tables 1 and 2). PCK *AVP*^{-/-} rats and Brattleboro controls had marked polyuria and a slight increase in plasma sodium concentration. Renal cAMP content was markedly reduced in the PCK *AVP*^{-/-} compared with

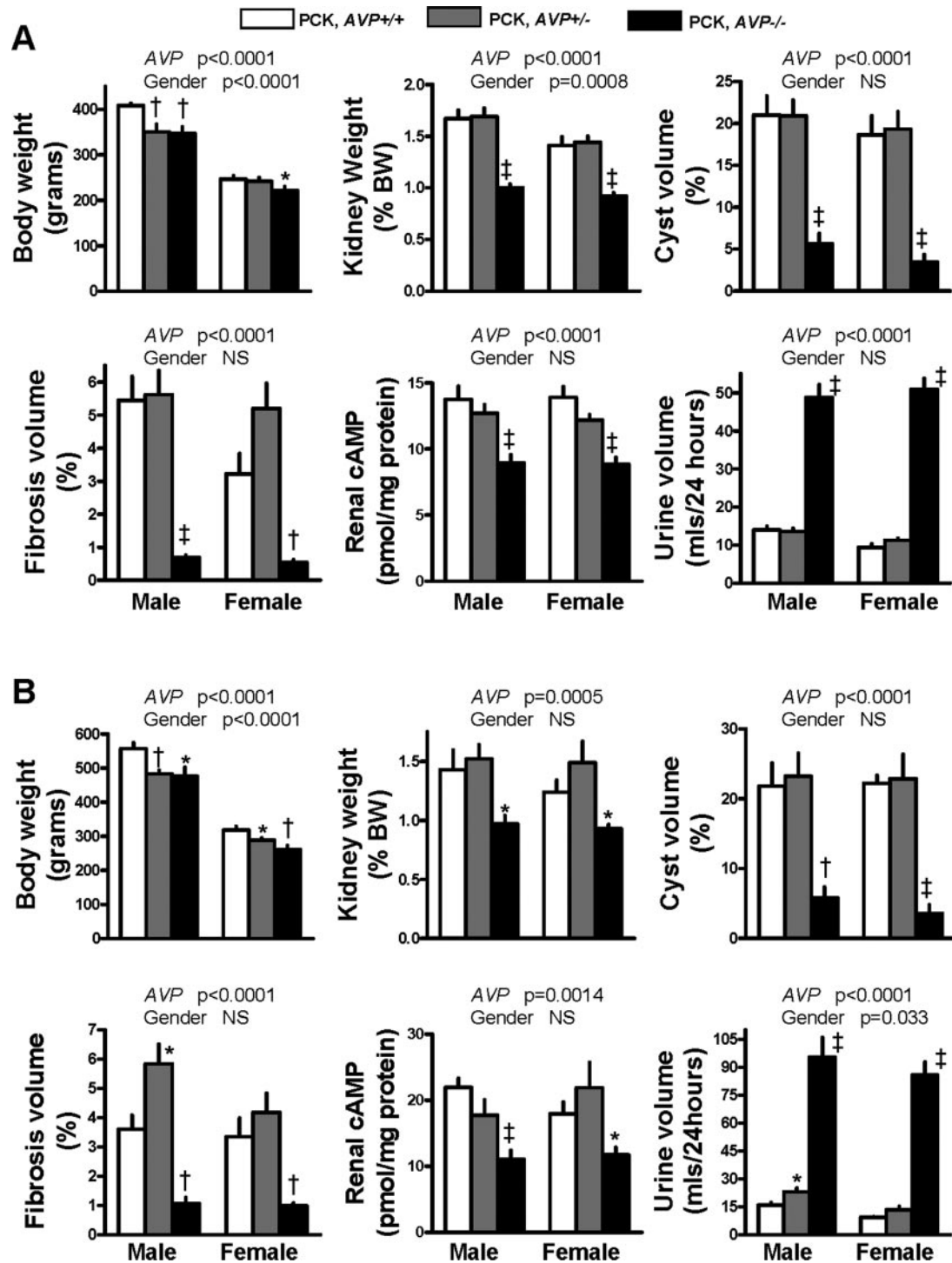


Figure 1. Body and kidney weights, indices of cystic disease severity, renal cAMP, and urine output, in 10-wk-old (A) and 20-wk-old (B) PCK *AVP*^{+/+}, PCK *AVP*^{+/-}, and PCK *AVP*^{-/-} rats. Two-way ANOVA *P* values for *AVP* gene and gender effect are shown above the bar graphs. **P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001 versus PCK *AVP*^{+/+} rats.

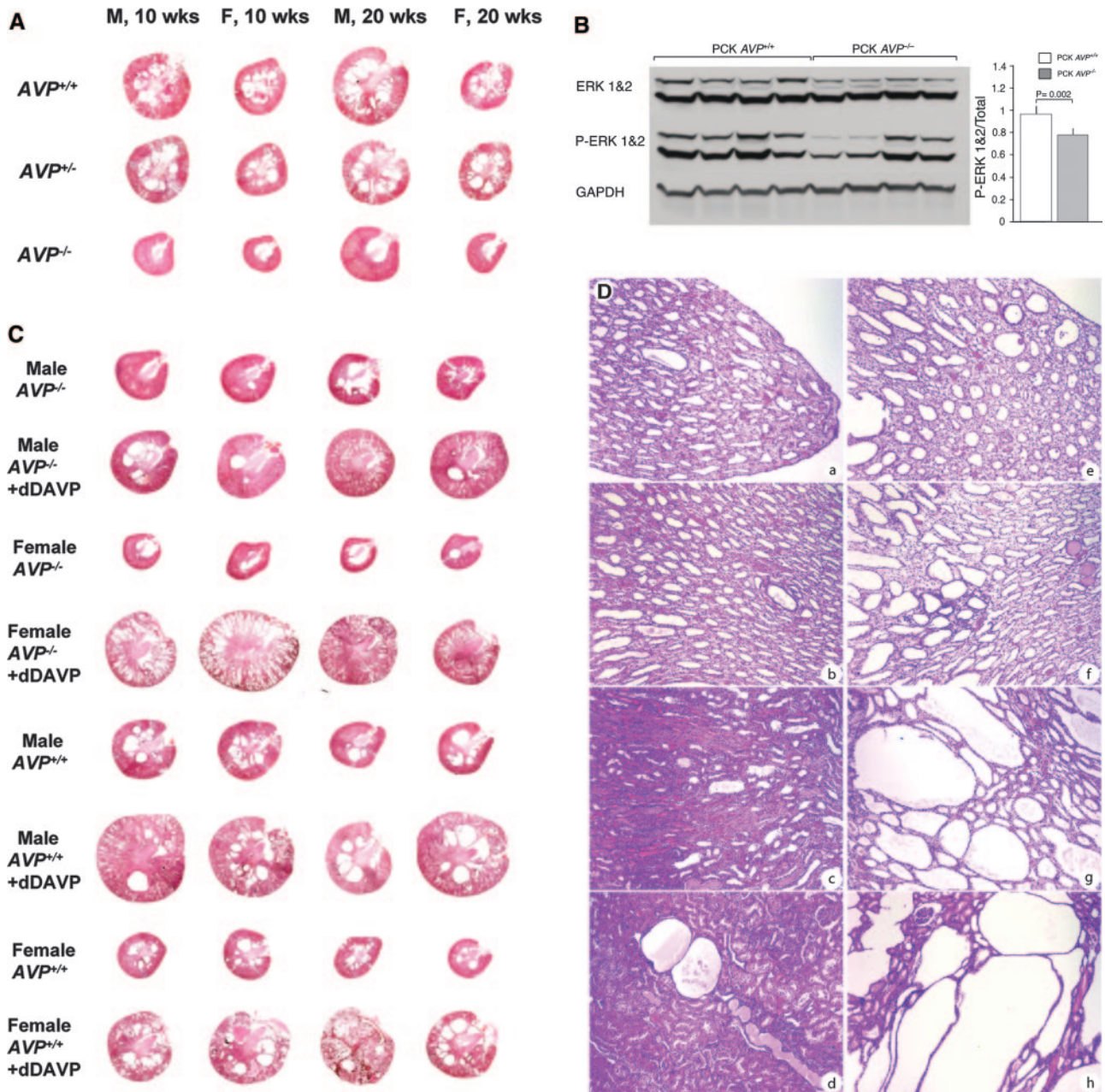


Figure 2. (A) Representative hematoxylin- and eosin-stained kidney sections from 10- and 20-wk-old male and female PCK AVP^{+/+}, PCK AVP^{+/-}, and PCK AVP^{-/-} rats. PCK AVP^{-/-} kidneys exhibit nearly complete protection from cystic disease. (B) Western blot of total and phosphorylated ERK1/2 and glyceraldehyde-3-phosphate dehydrogenase. ERK1/2 phosphorylation is significantly reduced in PCK AVP^{-/-} compared with PCK AVP^{+/+} kidneys. (C) Hematoxylin- and eosin-stained kidney sections from male and female PCK AVP^{-/-} and PCK AVP^{+/+} rats (*n* = 4 for each genotype and gender) treated continuously with dDAVP (10 ng/h per 100 g body wt) or vehicle alone between 12 and 20 wk of age. The administration of dDAVP recovered the cystic phenotype of the PCK AVP^{-/-} and aggravated the cystic disease of the PCK AVP^{+/+} rats. (D) Representative photomicrographs of papilla, inner medulla, outer medulla, and cortex from male PCK AVP^{-/-} rats treated continuously with vehicle (a through d) or dDAVP (e through h) between 12 and 20 wk of age. Note the mild focal dilation of collecting ducts in animals treated with vehicle alone. Administration of dDAVP results in diffuse dilation of collecting ducts, which is particularly prominent in the outer medulla and cortex. Magnification, ×100 (hematoxylin and eosin).

the PCK AVP^{+/+} rats. This was accompanied by decreased phosphorylation of extracellular signal-regulated kinase (ERK) and a marked reduction in kidney weight and renal cyst

and fibrosis volumes. No significant differences in liver or hepatic cyst or fibrosis volumes were detected, indicating that the protective effect of vasopressin deficiency is restricted to the

kidneys. These results are consistent with the renal protective effects of AVP V2 receptor antagonists (OPC31260 and OPC41061)^{3–5} and inhibition of vasopressin by forced water ingestion in the PCK rat.⁸

PCK $AVP^{+/-}$ rats, like PCK $AVP^{-/-}$ rats and Brattleboro controls, had slightly reduced body weights, but their renal cAMP levels, kidney weights, and cyst and fibrosis volumes were not lower than those in PCK $AVP^{+/+}$ rats (Figures 1 and 2A, Supplementary Tables 1 and 2). Male PCK $AVP^{+/-}$ rats had slightly higher urine outputs and lower urine osmolalities than PCK $AVP^{+/+}$ rats, particularly at 20 wk of age. This likely reflects a mild vasopressin deficiency that may become more evident with aging⁹ but is not sufficient to affect the renal concentration of cAMP or the development of PKD.

For confirmation that the protective effect of AVP deficiency on the development of PKD is due the lack of stimulation of the renal V2 receptors, PCK $AVP^{-/-}$, PCK $AVP^{+/+}$, and wild-type rats were treated with the V2 agonist 1-deamino-8-D-arginine vasopressin (dDAVP) administered *via* osmotic minipump at a dosage of 10 ng/h per 100 g body wt between 12 and 20 wk of age. This dosage is necessary to achieve urine osmolalities in Brattleboro rats similar to those observed in wild-type Sprague-Dawley rats.¹⁰ Administration of dDAVP to PCK $AVP^{-/-}$ reversed the polyuria, increased the renal concentration of cAMP, recovered the full cystic PCK phenotype as reflected by the kidney weights and cyst and fibrosis indices, and significantly increased the plasma blood urea nitrogen concentrations (Figures 2, C and D, and 3; Supplementary Table 3). Administration of dDAVP to wild-type rats at the dosage used in this study caused a slight but significant increase in renal mass per unit of body weight without inducing cystic changes or fibrosis. This is consistent with previous reports of selective AVP-induced hypertrophy of the medullary thick ascending limb in wild-type and Brattleboro rats.^{11,12} Consistent with the urine-concentrating defect and resistance to vasopressin associated with PKD,¹³ 24-h urine volumes were significantly higher and antidiuretic responses to dDAVP blunted in PCK $AVP^{+/+}$ compared with wild-type rats.

As shown in previous studies,^{3–5,14} the renal cystic disease in untreated PCK rats is more severe in males than in females. The cause of this dimorphism is not known. Our study suggests a possible relationship to gender-related differences in the secretion of AVP and its subsequent actions on the kidney. Release of AVP into the circulation in response to osmotic stimulation,¹⁵ plasma and urine AVP levels, V2 receptor density and AVP-dependent cAMP production in collecting ducts,^{16–20} antidiuretic effects of endogenous (water deprivation)²¹ and exogenous AVP,^{16–19} and AVP-dependent (or dDAVP-dependent) susceptibility to develop desoxycorticosterone acetate-salt hypertension^{16–19} all are higher in male than in female rats. The administration of dDAVP in our study eliminated the sexual dimorphism of PKD, possibly because the circulating concentration of dDAVP achieved in our study exceeded the range where estradiol is capable of modulating its antidiuretic action.^{16–19}

These observations may be relevant to patients with AD-

PKD. AVP secretion is more sensitive to osmotic stimuli,²² and plasma and/or urinary AVP levels^{23–25} and urine osmolality²⁶ are higher in men than in women. Whether men have a higher or a lower renal sensitivity to AVP than women is not clear, because studies on this issue are not in agreement.^{20,27} Higher AVP levels and gender differences in urine concentration may contribute to the faster rate of cystic expansion²⁸ and the earlier age of ESRD in men with ADPKD as observed in some^{29,30} but not all^{31,32} studies.

In summary, the results of this study indicate that AVP is a powerful modulator of cystogenesis. In the evolution from aquatic vertebrates to tetrapods, vasotocin in birds and vasopressin in mammals became essential for water homeostasis and survival.³³ To avoid dehydration, humans live under the tonic influence of circulating AVP and persistent activation of adenylyl cyclase in the distal nephron and collecting duct. Only for short periods of time, after drinking large volumes of liquid, do plasma AVP levels decrease enough to render the urine more dilute than plasma. During most of the day, cyst epithelial cells are persistently stimulated to proliferate and secrete fluid. This may be further enhanced by the renal concentrating defect and increased circulating levels of AVP that occur in PKD. These observations provide further support for clinical trials of V2 receptor antagonists in this disease.

CONCISE METHODS

Experimental Animals and Study Design

The use of PCK rats (maintained at the animal facilities of the Mayo Clinic, Rochester, MN) and Brattleboro rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) and the experimental protocols were approved by the Institutional Animal Care and Utilization Committee.

Genotyping

In PCK rats, the *Pkhd1* exon 36 is missing and genomic sequencing showed the mutation to be an A (labeled with VIC)→T (labeled with FAM) transversion at the –2 position of IVS35.⁶ In Brattleboro rats, there is a single nucleotide deletion (G, labeled with VIC, and N, labeled with FAM) in exon B of the *AVP* gene.⁷ Genomic DNA was extracted from rat tail using QIAamp DNA Mini kit (Qiagen, Valencia, CA). Genotyping for the *Pkhd1* substitution and a single base deletion in *AVP* was performed using commercially available custom-made TaqMan Genotyping assays. Real-time TaqMan PCR was performed according to the manufacturer's standard PCR. Twenty nanograms of total DNA was mixed with the 2× TaqMan Universal PCR Master Mix No AmpErase UNG and TaqMan Assay Mix to a final volume of 5 μ l. Each sample underwent 40 amplification cycles on an ABI. Two fluorogenic minor groove binder probes were used for each locus using the dyes 6-carboxyfluorescein (FAM; excitation, 494 nm) and VIC (excitation, 538 nm), which are easily differentiated in the Applied Biosystems Prism 7900HT PCR system. The resulting cluster plot showed strong fluorescent signals for each allele and clear separation between the three clusters, easily discriminating the homozygous and heterozygous genotypes.

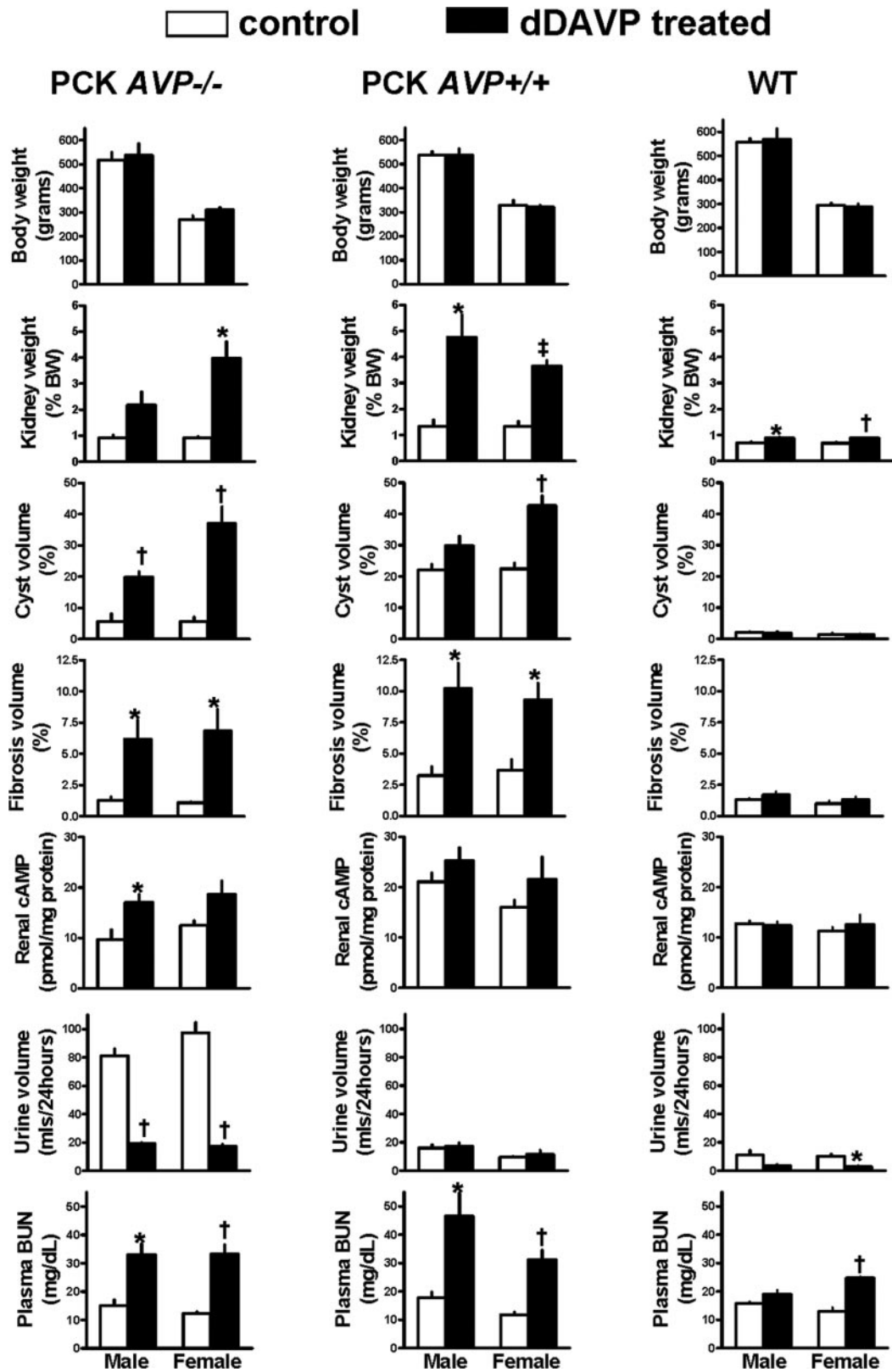


Figure 3. Effects of continuous subcutaneous administration of dDAVP (10 ng/h per 100 g body wt) or vehicle alone from 12 to 20 wk of age on body and kidney weights, urine output, indices of cystic disease severity, and laboratory parameters in PCK AVP^{-/-} and PCK AVP^{+/+} rats. The administration of dDAVP recovered the cystic phenotype of the PCK AVP^{-/-} and aggravated the cystic disease of the PCK AVP^{+/+} rats. **P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001 versus control rats receiving vehicle alone.

Experimental Protocol

The rats were maintained at the animal facilities of the Mayo Clinic. In some rats, osmotic minipumps (model 2ML4; Alzet, Palo Alto, CA) delivering vehicle alone or dDAVP at a rate of 10 ng/h per 100 g body wt were implanted subcutaneously through a small dorsal incision at 12 wk of age and replaced 4 wk later under anesthesia (ketamine 50 mg/kg and xylazine 10 mg/kg intraperitoneally). Twenty-four-hour urine outputs in metabolic cages and tail-cuff BP were obtained weekly on three consecutive weeks before rats were killed at 10 or 20 wk of age. After being killed, the rat were weighed and anesthetized with ketamine 50 mg/kg and xylazine 10 mg/kg, intraperitoneally. Blood was obtained by cardiac puncture for determination of serum electrolytes and blood urea nitrogen levels. The right kidney and the liver were placed into preweighed vials containing 10% formaldehyde in phosphate buffer (pH 7.4). The tissues were embedded in paraffin for histologic studies. The left kidneys were immediately frozen in liquid nitrogen for determination of cAMP.^{3–5}

Histomorphometric Analysis

Four-micron transverse tissue sections, including cortex, medulla, and papilla, were stained with hematoxylin-eosin to measure cyst volumes and with picosirius red stain for collagen to measure fibrosis. Image analysis procedures were performed with Meta-Morph software (Universal Imaging, West Chester, PA).^{3–5}

cAMP Content of Whole Kidneys

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, the supernatants were extracted with 3 Vol of water-saturated ether. After drying the aqueous extracts, the reconstituted samples were processed without acetylation using an enzyme immunoassay kit (Sigma-Aldrich, St. Louis, MO). The results were expressed in picomoles per milligram of protein.^{3–5}

Western Blot Analysis

Total ERK1/2 and phosphorylated ERK1/2 were measured by Western blot analysis using anti-ERK1/2 (C16) and anti-phosphorylated ERK1/2 (E4) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA).

Statistical Analyses

Comparisons between groups were made using one-way or two-way ANOVA with least significant difference comparisons of the means or *t* test as appropriate. Data are expressed as means ± SD.

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DISCLOSURES

None.

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