Increased Water Intake Decreases Progression of Polycystic Kidney Disease in the PCK Rat

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Renal enlargement in polycystic kidney disease (PKD) is caused by the proliferation of mural epithelial cells and transepithelial fluid secretion into the cavities of innumerable cysts. Arginine vasopressin (AVP) stimulates the proliferation of human PKD cells in vitro via cAMP-dependent activation of the B-Raf/MEK (MAPK/ERK kinase/extracellular signal-regulated kinase (ERK) pathway. ERK activity is elevated in cells that line the cysts in animals with PKD, and AVP receptor antagonists reduce ERK activity and halt disease progression. For suppression of the effect of AVP physiologically, water intake was increased in PCK rats, a model of PKD, and the effect on renal morphology, cellular mechanism, and function was determined. The addition of 5% glucose in the drinking water increased fluid intake approximately 3.5-fold compared with rats that received tap water. In PCK rats, increased water intake for 10 wk reduced urinary AVP excretion (68.3%), and urine osmolality fell below 290 mOsmol/kg. High water intake was associated with reduced renal expression of AVP V2 receptors (41.0%), B-Raf (15.4%), phosphorylated ERK (38.1%), and proliferating cell nuclear antigen–positive renal cells (61.7%). High water intake reduced the kidney/body weight ratio 28.0% and improved renal function. Taken together, these data demonstrate that water intake that is sufficient to cause persistent water diuresis suppresses B-Raf/MEK/ERK activity and decreases cyst and renal volumes in PCK rats. It is suggested that limiting serum AVP levels by increased water intake may be beneficial to some patients with PKD.

Mutations in the polycystic kidney disease (PKD) genes (PKD1, PKD2, and PKHD1) (1–4) disrupt intracellular Ca$^{2+}$ regulation and, thereby, transform renal epithelial cells into hyperplastic fluid-filled cysts. Epithelial cells that are isolated from cysts of human autosomal dominant (ADPKD) and autosomal recessive (ARPKD) kidneys have a lower basal intracellular Ca$^{2+}$ level compared with cells that are derived from normal renal tissue (5). cAMP accelerates the proliferation of ADPKD and ARPKD cells but has no mitogenic effect on normal renal tubule cells (6–8). The proliferative effect of cAMP in PKD cells is mediated by protein kinase A (PKA) stimulation of the MAPK/ERK kinase (MEK)/extracellular signal–regulated kinase (ERK) pathway through the intermediacy of B-Raf, a kinase that phosphorylates and activates MEK. By contrast, B-Raf is normally repressed in normal human kidney cells, and cAMP inhibits ERK activity and cell proliferation (9). Sustained reduction of intracellular Ca$^{2+}$ predisposes normal renal cells to cAMP-dependent stimulation of the B-Raf/MEK/ERK pathway and cell proliferation (6). Conversely, restoration of intracellular Ca$^{2+}$ concentration in cystic cells that are derived from human ADPKD and ARPKD kidneys rescues the normal antimitogenic response of cAMP (5).

Arginine vasopressin (AVP) is an important antidiuretic hormone that mediates its effect through the activation of vasopressin V2 receptors (AVP2V) and the subsequent stimulation of adenylyl cyclase and synthesis of cAMP (10). Normally, urine is concentrated to an osmolality that is greater than plasma. Day-to-day maintenance of urine output depends on appropriate plasma AVP levels to regulate osmotic water reabsorption by distal tubules and collecting ducts. Relatively normal plasma levels of AVP may be sufficient to stimulate cyst epithelial cell growth and renal enlargement in patients with PKD. Some patients have an intrinsic defect in the capacity to concentrate urine maximally, potentially leading to even greater levels of plasma AVP than normal (11–13).

In this study, we determined whether suppression of plasma AVP levels by increased water intake would be sufficient to slow PKD progression in PCK rats. In this model of PKD, orthologous to human ARPKD, mutations in Pkhd1 cause cysts to form in the collecting ducts (14). Many features of the disease in PCK rats resemble human ADPKD, such as focal development of cysts, although the pattern of inheritance is autosomal recessive. The results demonstrate that increased water intake...
that was sufficient to suppress the renal effects of AVP decreased MEK/ERK activity in the kidneys and slowed the progression of cystic disease in PCK rats.

Materials and Methods

PCK Rat Model

PCK rats that originally were derived from a strain of the Sprague-Dawley rats are maintained at the Education and Research Center of Animal Models for Human Diseases of Fujita Health University. These animals have been characterized previously (15). PCK rats and normal Sprague-Dawley (+/+; Charles River Japan Inc., Kanagawa, Japan) rats were allowed free access to water and food throughout the study. PCK and +/+ rats were randomly assigned to either the control group (normal tap water) or high water intake (HWI) group (water that contained 5% glucose) and treated as such from 4 to 14 wk of age. Twenty-four-hour samples were collected in metabolic cages after 13.5 wk to determine urine volume, water intake volume, and food consumption. At 14 wk of age, rats were anesthetized with pentobarbital sodium (Scherling-Plough Corp., Kenilworth, NJ), and both kidneys were removed rapidly, causing exsanguination. Total kidney weight was measured, and then the left kidney was homogenized in lysis buffer to extract proteins and the right kidney was sectioned and immersed in 4% paraformaldehyde. Western Blot Analysis

Kidney lysate samples were prepared for immunoblot analysis (16,17). Contents of lysis buffer (TLB buffer) were 20 mmol/L Tris (pH 7.4), 137 mmol/L NaCl, 25 mmol/L β-glycerophosphate, 2 mmol/L EDTA, 1 mmol/L sodium orthovanadate, 2 mmol/L dithiothreitol, 1 mmol/L PMSF, 5 μg/ml aprotinin, and 5 μg/ml leupeptin with 1% Triton X-100. Membrane-blotted lysates (20 μg protein/lane) were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. Membranes were blocked with 5% milk in TBS-T (20 mmol/L Tris-HCl [pH 8.0], 137 mmol/L NaCl, and 0.05% Tween 20) for 1 h at room temperature and then incubated overnight at 4°C in primary antibody (1:3000) for phosphorylated ERK (P-ERK) or proliferating cell nuclear antigen (PCNA) overnight at 4°C. Sections were rinsed with PBS, incubated with biotinylated secondary antibody, and then incubated with streptavidin conjugated to peroxidase (Histofine; Nichirei Biosciences, Tokyo, Japan). Immune reaction products were developed using 3,3’-diaminobenzidine. Cystic surface area was measured from 10 random fields (×100 magnification) of hematoxylin-eosin–stained sections of renal cortex (n = 3 PCK rats). Cyst area (μ²/field) was measured by a naive observer using LUZEX software (Kideko Co. Ltd., Tokyo, Japan). ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon International, Temecula, CA) was used to detect apoptotic cells. Apoptotic cells and P-ERK or PCNA-positive nuclei were counted from 500 to 600 cells (×400 magnification) per three thin sections from either PCK or +/+ rat kidneys.

Antibodies

Primary antibodies to B-Raf (F-7, SC-5284), ERK1,2 (K-23, SC-94), P-ERK (E-4, SC-7383), and Raf-1 (C-12, SC-133) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) for immunoblot analysis.

Table 1. Effect of HWI on urine volume and osmolality and urinary AVPa

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>n</th>
<th>Water Intake (mL)</th>
<th>Urine Volume (mL)</th>
<th>Urine Osmolarity (mOsm/kg H₂O)</th>
<th>Urinary AVP (pg/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCK</td>
<td>CONT</td>
<td>8</td>
<td>18 ± 1</td>
<td>18 ± 2</td>
<td>1088 ± 96</td>
<td>257 ± 22</td>
</tr>
<tr>
<td>PCK</td>
<td>HWI</td>
<td>10</td>
<td>68 ± 5b</td>
<td>58 ± 5b</td>
<td>232 ± 47b</td>
<td>114 ± 46b</td>
</tr>
<tr>
<td>+/+</td>
<td>CONT</td>
<td>8</td>
<td>10 ± 5</td>
<td>9 ± 1</td>
<td>1498 ± 130</td>
<td>160 ± 19</td>
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<tr>
<td>+/+</td>
<td>HWI</td>
<td>8</td>
<td>94 ± 26c</td>
<td>91 ± 10b</td>
<td>197 ± 73b</td>
<td>88 ± 38</td>
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<td>Female</td>
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<tr>
<td>PCK</td>
<td>CONT</td>
<td>10</td>
<td>18 ± 3</td>
<td>16 ± 2</td>
<td>982 ± 74</td>
<td>435 ± 83</td>
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<tr>
<td>PCK</td>
<td>HWI</td>
<td>8</td>
<td>63 ± 9b</td>
<td>49 ± 8b</td>
<td>284 ± 52b</td>
<td>111 ± 59b</td>
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<tr>
<td>+/+</td>
<td>CONT</td>
<td>7</td>
<td>13 ± 5</td>
<td>12 ± 1</td>
<td>1599 ± 211</td>
<td>335 ± 49</td>
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<td>HWI</td>
<td>10</td>
<td>107 ± 10b</td>
<td>80 ± 11b</td>
<td>131 ± 25b</td>
<td>31 ± 4b</td>
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</tbody>
</table>

aAddition of 5% glucose to drinking water increased water intake 3.5-fold in PCK rats and 8.2-fold in normal Sprague-Dawley (+/+ ) rats (composites of male and female rats). Rats drink more water that contained glucose because they have a preference for sweet fluid (33). Urine volume increased accordingly. High water intake (HWI) decreased urine osmolalities below 290 mOsmol/kg H₂O. Urinary arginine vasopressin (AVP), an indicator of plasma AVP levels, decreased significantly in both PCK and +/+ rats with HWI. Statistical differences between HWI and control (CONT) rats (either +/+ or PCK) were determined by one-way ANOVA.

bP < 0.01.
cP < 0.05.
Secondary antibodies conjugated to horseradish peroxidase were goat anti-rabbit IgG (SC-2054) and rabbit anti-mouse IgG (SC-2055) from Santa Cruz Biotechnology. Primary antibodies to AVPV2R (AB1797P; Chemicon International), PCNA (P8825; Sigma Chemical, St. Louis, MO) and P-ERK (M5670; Sigma) were used for immunohistochemistry. Secondary antibody, conjugated to biotin, for immunohistochemistry studies was rabbit anti-mouse IgG/IgA/IgM (HISTOFINE 426032) obtained from Nichirei (Tokyo, Japan).

**Measurement of Urine AVP, Creatinine and Osmolality, and Serum Urea Nitrogen**

Urine AVP levels were measured with a Correlate-EIA TM arg8–Vasopressin Enzyme Immunoassay Kit (cat. no. 900-017; Assay Designs, Inc., Ann Arbor, MI). Urine creatinine measurements were determined by a colorimetric microplate assay kit (Kit CR01; Oxford Biomedical Research, Inc., Oxford, MI). For an AVP concentration below the level of detection (<3.39 pg/ml), a value of 3.38 pg/ml was used for the calculation of AVP per creatinine concentration (pg

**Figure 1.** Effect of high water intake (HWI) on kidney weight of PCK and normal rats. (A) The addition of 5% glucose to the drinking water did not affect body weight of either the PCK or normal Sprague-Dawley (+/+) rats. (B) HWI caused a significant decrease in total kidney weight, represented as percentage of body weight in both male and female PCK rats. By contrast, HWI had no effect on total kidney weight of +/+ rats. Statistical differences between HWI and control (CONT) rats (either +/- or PCK) were determined by one-way ANOVA; **P < 0.01.

**Figure 2.** Effect of HWI on serum urea nitrogen (SUN) in PCK and normal rats. Male PCK rats at 14 wk of age had an elevated SUN compared with age-matched +/+ rats. HWI decreased SUN to control levels in PCK rats. The small but significant decrease in SUN in +/+ rats with HWI is consistent with increased urea excretion as a result of elevated fluid flow in the distal nephron. Statistical differences between HWI and control (CONT) rats (either +/+ or PCK) were determined by one-way ANOVA; *P < 0.05, **P < 0.01.

**Figure 3.** Effect of HWI on renal cyst development in male and female PCK rats. Micrographs of representative kidney sections, stained with hematoxylin-eosin, were taken at the same magnification from a PCK male rat with normal water intake (A), a PCK male rat with HWI (B), a PCK female rat with normal water intake (C), and a PCK female rat with HWI (D). (E) Surface area of cysts (mean ± SE) from representative sections of male and female PCK kidneys was measured by morphometric analysis. Increased water intake decreased cyst area 60 and 50% in male and female PCK kidneys, respectively. Comparison between HWI and CONT rats (male or female), **P < 0.01.
AVP/mg creatinine). Osmolality was determined with a freezing-point osmometer (Osmostat OM-6040; Kyoto Diiichi Kagaku, Kyoto, Japan). Serum urea nitrogen (SUN) determinations were performed using a colorimetric assay kit using a Urease-Indophenol method (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Statistical Analyses
Data represent mean and SE. Statistical significance was determined by one-way ANOVA and Student-Newman-Keuls post test for multiple comparisons or unpaired t-test for comparison between control and treated animals.

Results
In PCK rats and normal Sprague-Dawley (+/+ ) rats that were offered water that contained 5% sucrose increased fluid intake from 64 ± 5 ml (n = 6) to 155 ± 8 ml (n = 15). There was no effect of HWI on body weight, total kidney weight, or kidney weight/body weight. Increased water intake for 10 wk decreased SUN from 38.7 to 26.3 mg/dl in the PCK male rats, a level that was similar to that of control (+/+ ) rats that drank tap water. Therefore, increased fluid intake that was sufficient to cause water diuresis and reduce urinary excretion of AVP, ostensibly as a result of reduced plasma AVP levels, slowed the progression of cystic disease in PCK rats. By contrast, in Hans SPRD (Cy/+ ) rats, which develop cysts in the proximal tubules (a segment that lacks AVPV2R), HWI caused polyuria and decreased urine osmolality but failed to reduce the severity of cystic disease (Table 2). Therefore, polyuria and decreased urine osmolality per se do not account for the striking reduction in kidney size that is caused by HWI.

Previously, Gattone et al. (20) reported overexpression of mRNA for AVPV2R in kidneys of PCK rats and C57BL/6j-cpk/
cPK mice, another model for recessive PKD (12). In this study, we confirmed that protein expression of AVPV2R was elevated in PCK kidneys compared with control kidneys (Figure 4). Therefore, overexpression of AVPV2R in the epithelial cells of collecting duct cysts could contribute to persistently high levels of cAMP (21). It is interesting that HWI normalized AVPV2R expression in the PCK kidneys (Table 3).

The levels of a 95-kD isoform of B-Raf and P-ERK were elevated in kidneys of male and female PCK rats compared with gender-matched +/+ rats (Figure 4, Table 3), consistent with previous findings (22). HWI decreased the level of P-ERK 33% in male and 41% in female rats (composite average 38.1%) but had no effect on P-ERK levels in +/+ rats. Furthermore, increased water intake reduced B-Raf abundance 19.7% in PCK male kidneys. There also was a small reduction (11.1%) in B-Raf abundance in PCK female kidneys; however, this difference was not statistically significant. Immunohistochemical analysis revealed that HWI decreased the number of cells that stained positive for PCNA, a proliferation marker, by approximately 60% (male and female; Figure 5). The number of cells (% of cells per section) that stained positive for P-ERK was reduced 52% in PCK male rats and 44% in PCK female rats (Figure 6), confirming the observations made by immunoblot analysis. Moreover, the number of apoptotic cells (%) in the kidneys of PCK rats was elevated compared with +/+ rats (Figure 7). HWI reduced the number of apoptotic cells in both male and female PCK kidneys. Taken together, these data show that the renal protective effects of increased water intake in PKD likely were mediated by reductions in AVPV2R expression, B-Raf abundance, and activity of the B-Raf/MEK/ERK pathway.

**Discussion**

We determined whether reduction of plasma AVP levels by the levels of a 95-kD isoform of B-Raf and P-ERK were elevated in kidneys of male and female PCK rats compared with gender-matched +/+ rats (Figure 4, Table 3), consistent with previous findings (22). HWI decreased the level of P-ERK 33% in male and 41% in female rats (composite average 38.1%) but had no effect on P-ERK levels in +/+ rats. Furthermore, increased water intake reduced B-Raf abundance 19.7% in PCK male kidneys. There also was a small reduction (11.1%) in B-Raf abundance in PCK female kidneys; however, this difference was not statistically significant. Immunohistochemical analysis revealed that HWI decreased the number of cells that stained positive for PCNA, a proliferation marker, by approximately 60% (male and female; Figure 5). The number of cells (% of cells per section) that stained positive for P-ERK was reduced 52% in PCK male rats and 44% in PCK female rats (Figure 6), confirming the observations made by immunoblot analysis. Moreover, the number of apoptotic cells (%) in the kidneys of PCK rats was elevated compared with +/+ rats (Figure 7). HWI reduced the number of apoptotic cells in both male and female PCK kidneys. Taken together, these data show that the renal protective effects of increased water intake in PKD likely were mediated by reductions in AVPV2R expression, B-Raf abundance, and activity of the B-Raf/MEK/ERK pathway.

**Table 3. Effects of HWI on AVPV2 expression and B-Raf/MEK/ERK activity in PCK and +/+ kidneys**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>AVPV2R</th>
<th>B-Raf</th>
<th>P-ERK</th>
<th>ERK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCK</td>
<td>CONT</td>
<td>1.48 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.70 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13 ± 0.03</td>
</tr>
<tr>
<td>PCK</td>
<td>HWI</td>
<td>0.79 ± 0.09</td>
<td>1.18 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.80 ± 0.42&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>+/+</td>
<td>CONT</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>+/+</td>
<td>HWI</td>
<td>0.65 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.00 ± 0.00</td>
<td>1.14 ± 0.11</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PCK</td>
<td>CONT</td>
<td>2.11 ± 0.25</td>
<td>1.44 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.47 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99 ± 0.07</td>
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<tr>
<td>PCK</td>
<td>HWI</td>
<td>1.33 ± 0.04&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.28 ± 0.1</td>
<td>2.65 ± 0.17&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.99 ± 0.05</td>
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<tr>
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<td>CONT</td>
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<tr>
<td>+/+</td>
<td>HWI</td>
<td>0.90 ± 0.20</td>
<td>1.01 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.99 ± 0.03</td>
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</tbody>
</table>

<sup>a</sup>Summary of the fold changes in renal expression of AVPV2R, 95-kD isoform of B-Raf, P-ERK, and total ERK in PCK and +/+ rats (male and female) treated with normal water intake (CONT) or HWI. These data were obtained from animals listed in Table 1. MEK, MAPK/ERK kinase; AVPV2R, vasopressin V2 receptor; P-ERK, phosphorylated extracellular signal–regulated kinase.

Comparisons between PCK and +/+ rats, <sup>b</sup><i>P</i> < 0.05, <sup>c</sup><i>P</i> < 0.01; comparison between HWI and control (CONT) rats (either +/+ or PCK), <sup>d</sup><i>P</i> < 0.01, <sup>e</sup><i>P</i> < 0.05.
basis for the cAMP-mitogenic phenotype in PKD seems to be due to dysregulation of intracellular Ca\(^{2+}\) secondary to mutations in the PKD genes (5,6). ARPKD and ADPKD cyst-derived cells have [Ca\(^{2+}\)]\(_i\) that is lower than tubule cells derived from noncystic regions of ADPKD kidneys or normal human kidneys. Therefore, cyst-lining cells are associated with lower basal [Ca\(^{2+}\)]\(_i\), and cAMP-dependent activation of B-Raf/MEK/ERK and cell proliferation. The cellular pathway for Ca\(^{2+}\) regulation of the cAMP-mitogenic phenotype remains to be established, but the phosphatidylinositol 3-kinase/Akt pathway seems to be involved (5,6). Basal Akt activity was found to be reduced in ADPKD cells compared with normal human kidney cells, and agents that increased [Ca\(^{2+}\)]\(_i\) also stimulated Akt and blocked cAMP-dependent B-Raf activation of the MEK/ERK pathway. Recently, Boca et al. (29) showed that the expression of full-length polycystin-1 in renal cells increased the activity of phosphatidylinositol 3-kinase/Akt signaling pathway, supporting a role for this pathway in cystogenesis.

Normal plasma AVP levels which respond to fluctuations in extracellular fluid osmolality are likely to maintain renal intracellular cAMP at levels that activate the MEK/ERK pathway and proliferation of PKD cystic epithelial cells, promoting cystogenesis. However, increased water intake (HWI) decreased the percentage of apoptotic cells by 52 and 65% in the male and female PCK rats, respectively. Comparing between HWI- and CONT-treated PCK rats (male or female), **P < 0.01. 

Figure 5. Effect of HWI on cell proliferation in PCK and +/+ kidneys. Representative kidney sections from PCK and +/+ rats (male and female) that were treated with normal water intake (CONT) or HWI. Sections were stained with an antibody to proliferating cell nuclear antigen (PCNA), a proliferation marker. Micrographs show PCNA-positive nuclei in cells of kidney sections from PCK-CONT (A), PCK-HWI (B), +/+ CONT (C), and +/+ HWI rats (D). (E) Number of cells (percentage of total) that were positive for PCNA. Kidney sections of +/+ rats had very few PCNA-positive cells. By contrast, approximately 40% of the cells in the PCK kidney stained positive for PCNA. Increased water intake decreased the percentage of proliferating cells in the kidneys of PCK rats. Comparison between HWI- and CONT-treated PCK rats (male or female), *P < 0.05, **P < 0.01.

Figure 6. Effect of HWI on P-ERK-positive cells in PCK and +/+ kidneys. Number of cells (percentage of total) positive for P-ERK was measured from micrographs of kidney sections of PCK and +/+ rats (male and female) that were treated with CONT or HWI. Male and female PCK rats on HWI had a significant reduction in the number of cells that stained positive for P-ERK, indicating that the renal MEK/ERK activity was reduced by increased water intake. Comparison between HWI- and CONT-treated PCK rats (male or female), *P < 0.05.

Figure 7. Effect of HWI on cellular apoptosis in PCK and +/+ kidneys. The number of apoptotic cells (percentage of total) in kidney sections of CONT- and HWI-treated PCK and +/+ rats was determined using a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling method. There was a striking elevation in the percentage of apoptotic cells in the PCK kidneys compared with +/+ kidneys. Increased water intake (HWI) decreased the percentage of apoptotic cells 52 and 65% in the male and female PCK rats, respectively. Comparison between HWI- and CONT-treated PCK rats (male or female), **P < 0.01.
and kidney enlargement. Several rodent models of PKD, including the PCK rat, have been reported to have elevated renal cAMP levels (12,21). Gattone and Torres (reviewed by Torres [13]) found that novel AVPV2R antagonists OPC-31261 and OPC-41061 (Tolvaptan; Otsuka Pharmaceutical, Tokyo, Japan) reduced renal cAMP levels and halted PKD progression in four different animal models, including PKD2−/− mice, as well as PCK rats (13,20,28). Treatment with OPC-41061 caused a concomitant reduction in renal levels of B-Raf and P-ERK (22). On the basis of these studies, the capacity for OPC-41061 to reduce PKD progression is being examined in clinical trials (30).

As with other models of PKD, PCK rats are characterized by tubular epithelial cell hyperplasia and increased rates of apoptosis, hallmarks of human PKD (31). We used immunohistochemistry to evaluate the effect of HWI on the proliferation and apoptosis of cyst-lining cells in PCK rat kidneys. The number of cells that stained positive for PCNA, a mitotic indicator, was measured in thin kidney sections from PCK rats. Consistent with a reduction in cyst area and kidney weight, HWI decreased cell proliferation of cyst epithelial cells (PCNA-positive cells decreased approximately 60%; Figure 5E). Inhibition of cell proliferation was associated with decreased levels of B-Raf and P-ERK, suggesting that increased water intake diminished the renal activity of the MEK/ERK pathway. Moreover, the number of apoptotic cells in the cyst epithelium of water-loaded PCK rats was significantly reduced compared with control-treated PCK rats (Figure 7). Male PCK rats at 14 wk of age were found to have increased SUN. Elevated SUN was corrected by increased water intake, demonstrating that suppression of plasma AVP decreased renal cyst formation and improved renal function.

In a retrospective analysis of patients with chronic renal insufficiency, Hebert et al. (32) found that high urine volume was associated with a faster decline of GFR. The authors suggested that high urine volume and low urine osmolality may be risk factors for the progression of renal disease, including PKD. Conversely, an inability to concentrate urine as kidney function declines could account for the high urine volume in their study. Therefore, it is impossible to establish a cause–effect relationship between water intake and the decline in GFR on the basis of their results. In this study, we found that high fluid intake slowed the progression of PKD and significantly improved renal function in the PCK rats. On the basis of these results, we suggest that increased water intake may be beneficial to patients with PKD.

Conclusion

This study provides evidence for a central role of AVP in cAMP-dependent activation of the B-Raf/MEK/ERK pathway in cyst-lining cells and renal cyst enlargement. Increased water intake that was sufficient to cause a sustained reduction in plasma AVP levels decreased AVPV2R expression, reduced ERK activity and renal cell proliferation, and slowed PKD progression in PCK rats. We propose that sustained hydration by increased water intake may be beneficial to some patients with PKD by limiting the detrimental effects of AVP on renal cyst growth.

Acknowledgments

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References
