Mouse model of inducible nephrogenic diabetes insipidus produced by floxed aquaporin-2 gene deletion

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Yang, Baoxue, Dan Zhao, Liman Qian, and A. S. Verkman. Mouse model of inducible nephrogenic diabetes insipidus produced by floxed aquaporin-2 gene deletion. Am J Physiol Renal Physiol 291: F465–F472, 2006. First published January 24, 2006; doi:10.1152/ajprenal.00494.2005.—Transgenic mouse models of defective urinary concentrating ability produced by deletion of various membrane transport or receptor proteins, including aquaporin-2 (AQP2), are associated with neonatal mortality from polyuria. Here, we report an inducible mouse model of AQP2 gene deletion with severe polyuria in adult mice. LoxP sequences were inserted into introns 1 and 2 in the mouse AQP2 gene by homologous recombination in embryonic stem cells. Mating of germ-line AQP2-loxP mice with tamoxifen-inducible Cre-expressing mice produced offspring with inducible homozygous Cre-AQP2-loxP, which had a normal phenotype. Tamoxifen injections over 10 days resulted in AQP2 gene expression in homozygous mice in which loxP sites were introduced in introns of the mouse AQP2 gene. The mice developed severe polyuria in adult mice. LoxP sequences were inserted into introns 1 and 2 in the mouse AQP2 gene by homologous recombination in embryonic stem cells. Mating of germ-line AQP2-loxP mice with tamoxifen-inducible Cre-expressing mice produced offspring with inducible homozygous Cre-AQP2-loxP, which had a normal phenotype. Tamoxifen injections over 10 days resulted in AQP2 gene expression in homozygous mice in which loxP sites were introduced in introns of the mouse AQP2 gene. The mice developed severe polyuria in adult mice.

METHODS

Generation of AQP2 conditional knockout mice. Based on mouse AQP2 gene structure (40), a gene replacement targeting vector was constructed as shown in Fig. 1A. The vector contained a 5-kb mouse genomic DNA fragment containing exons 1–3 of the AQP2 gene. Exon 2 and a Pol2neo/pA selection cassette were flanked by loxP sites for conditional gene deletion and positive selection with G418, and a PGKk cassette was inserted at the 3′-end of the AQP2 targeting sequence for negative selection with FIAU. The vector was linearized at a unique NsiI site and electroporated into CB1–4 embryonic stem (ES) cells. Transfected ES cells were selected with G418 and FIAU for 7 days, yielding 4 targeted clones of 96 doubly resistant colonies on PCR screening using a sense primer specific for the neomycin resistance gene (NeoR) and an antisense primer specific for the AQP2 gene. ES cells were injected into a unique NsiI site and electroporated into CB1–4 embryonic stem (ES) cells. Transfected ES cells were selected with G418 and FIAU for 7 days, yielding 4 targeted clones of 96 doubly resistant colonies on PCR screening using a sense primer specific for the neo cassette (5′-CTGCAGTTCCACCGCCTAGATGCAAT-3′) and an antisense primer specific for the AQP2 gene (5′-GCAAAAGCTCTGAGAGCCCGC-3′) located beyond the 3′-end of the construct. Homologous recombination was confirmed by Southern blot analysis using a 1-kb genomic fragment as probe as indicated in Fig. 1A. ES cells were injected into PC2.5-day, 8-cell morula stage CD1 zygotes, cultured overnight to blastocysts, and transferred to pseudopregnant B6D2 females. Offspring were genotyped by PCR followed by Southern blot analysis using a 1-kb genomic fragment as probe as indicated in Fig. 1A. ES cells were injected into PC2.5-day, 8-cell morula stage CD1 zygotes, cultured overnight to blastocysts, and transferred to pseudopregnant B6D2 females. Offspring were genotyped by PCR followed by Southern blot analysis using a 1-kb genomic fragment as probe as indicated in Fig. 1A. ES cells were injected into PC2.5-day, 8-cell morula stage CD1 zygotes, cultured overnight to blastocysts, and transferred to pseudopregnant B6D2 females. Offspring were genotyped by PCR followed by Southern blot analysis using a 1-kb genomic fragment as probe as indicated in Fig. 1A.

AQUAPORIN-2 (AQP2) IS THE ANTIDIURETIC HORMONE (VASOPRESSIN)-REGULATED WATER CHANNEL EXPRESSED IN THE MAMMALIAN KIDNEY COLLECTING DUCT. Apical plasma membrane AQP2 targeting is regulated by a vesicular transport mechanism in which vasopressin acting through cAMP causes the exocytic insertion of AQP2 (1). AQP2 is of clinical importance in acquired disorders of urinary concentrating function, both in nephrogenic diabetes insipidus (NDI), as produced by lithium therapy (20), and the syndrome of inappropriate antidiuretic hormone (SIADH) (2, 26). AQP2 is expressed in the renal medulla and is constitutively upregulated by 10.220.33.1 on June 11, 2017 http://ajprenal.physiology.org/ Downloaded from http://ajprenal.physiology.org/ by 10.220.33.1 on June 11, 2017 http://ajprenal.physiology.org/ by 10.220.33.1 on June 11, 2017 http://ajprenal.physiology.org/ by 10.220.33.1 on June 11, 2017
Cre/Esr1 transgenic mice (Jackson Laboratories, Bar Harbor, ME) (10) to yield mice heterozygous for both genes. The second crossing of heterozygous mice yielded homozygous floxed AQP2 mice with the Cre/Esr1 gene. Protocols were approved by University of California San Francisco Committee on Animal Research.

**Induction of AQP2 gene deletion.** A stock solution of tamoxifen (4-hydroxytamoxifen, Sigma, St. Louis, MO) was diluted in corn oil to 5 mg/ml. Adult mice (age 5–8 wk) were given intraperitoneal injections of the tamoxifen suspension (0.1 ml of 5 mg/ml) over 10 days.

**Southern and Northern blot analysis.** AQP2 gene targeting and deletion were confirmed by Southern hybridization in which 10 μg of genomic DNA were digested with ApaI, electrophoresed, transferred to a nylon+ membrane (Amersham Biosciences, Piscataway, NJ), and hybridized with a 1-kb genomic fragment (indicated in Fig. 1A). For Northern blot analysis, total RNA from kidney, inner ear, colon, testes, and tail from AQP2<sup>floxed</sup> mice (flox), digested with ApaI and probed as indicated in A. Where indicated, mice were treated for 10 days with tamoxifen (4OH-TM).

**Immunoblot analysis**

Kidney tissue was fixed in Bouin’s fixative (decalcified), testis, vas deferens, and colon tissue samples were fixed with 4% paraformaldehyde in PBS for 4 h, infiltrated with 30% sucrose in PBS overnight, frozen in OCT with liquid nitrogen, and cut into 3-μm-thick sections using a cryostat. Tissues were incubated using a rabbit polyclonal antibody against the 30-amino acid COOH terminus of AQP2 as described (40). Immunoblot analysis of the tissue homogenate was carried out with the same polyclonal serum. Tissues were homogenized with a glass Dounce homogenizer in 250 mM sucrose containing 1 mM EDTA, 20 μg/ml PMSF, 1 μg/ml pepstatin A, and 1 μg/ml leupeptin (pH 7.4), and centrifuged at 4,000 g for 15 min to remove whole cells, nuclei, and mitochondria. Total protein was assayed in the supernatant fractions using a Bio-Rad DC protein assay kit (Bio-Rad, Richmond, CA) and loaded on a 12% SDS-PAGE gel (10 μg/lane). Proteins were transferred to polyvinylidene difluoride membranes (Gelman Scientific, Ann Arbor, MI) and immunoblotted by standard procedures (40).

**Fluorescence-based real-time RT-PCR.** Total RNA from whole mouse kidney was isolated by homogenization in TRIzol reagent (Invitrogen), and mRNA was extracted using an Oligotex mRNA mini-kit (Qiagen, Valencia, CA). cDNA was reverse transcribed from mRNA with oligo(dT) (SuperScript II preamplification kit, Invitrogen). Fluorescence-based real-time RT-PCR was carried out using the LightCycler instrument (Roche Diagnostics, Indianapolis, IN) with a LightCycler FastStart DNA Master<sup>™</sup> SYBR Green I kit. Primers were designed, and real-time PCR was carried out as described previously (11). β-Actin was used as the reference gene, and pooled wild-type cDNA as the calibrator. Data are reported as normalized, calibrated ratios in which all samples were normalized to the reference gene.

**Kidney histology.** Kidney tissue was fixed in Bouin’s fixative overnight, embedded in paraffin, and cut into 3-μm-thick sections.

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**Fig. 1.** Gene targeting strategy for conditional aquaporin-2 (AQP2) gene knockout. A: top, organization and restriction map of the mouse AQP2 gene. Rectangles indicate exon segments, with coding regions shaded. Middle (2 lines): targeting strategy for floxed AQP2 gene deletion. Homologous recombination results in replacement of the indicated segments (thick lines) of the AQP2 gene. Exon 2 and a 1.8-kb polII-neo-selectable marker were flanked by loxP sites (arrowheads). Bottom: AQP2 gene structure after conditional deletion by Cre-recombinase. Probe used for Southern blot analysis is indicated ("probe") along with expected sizes of hybridized fragments after ApaI digestion. B: Southern blot of genomic DNA from kidney of wild-type (WT) mouse and of +4OH-TM.

**Fig. 2.** AQP2 gene expression after Cre-induced deletion of the floxed AQP2 gene. A: Northern blot of indicated tissues from tamoxifen-treated WT and AQP2<sup>floxed</sup> mice (flox) probed with the mouse AQP2 coding sequence. B: RT-PCR amplification of cDNA from indicated tissues with primers amplifying the full-length AQP2 coding region. C: immunoblot analysis using purified polyclonal AQP2 antibody. def., Deferens.
Kidney sections, including cortex, medulla and papilla, were stained with hematoxylin and eosin.

Urinary concentrating studies. Urine samples were collected by placing mice on a wire mesh platform in a clean glass beaker until spontaneous voiding was observed. In some experiments, urine samples were obtained from the same mice under basal conditions (unrestricted access to food and water), and after 18-h deprivation of food and water. Twenty-four-hour urine output and water consumption were measured in metabolic cages adapted for mice (Harvard Apparatus, Holliston, MA). Blood samples were collected in heparinized glass tubes by puncture of the periorbital venous sinus. Plasma was separated from blood cells by centrifugation. Urine osmolality was measured by freezing point osmometry (microosmometer, Precision Systems, Natick, MA). Urine and plasma chemistries were measured by the University of California San Francisco Clinical Chemistry Laboratory.

Statistical analysis. Statistical analysis was performed using Student’s t-test.

**RESULTS**

Figure 1A shows the gene-targeting strategy for generation of an inducible mouse model of AQP2 gene deletion. LoxP sequences (arrowheads) were inserted in intronic segments in the AQP2 gene, as well as Pol2neobpA and PGK-tk selection cassettes. Cre-recombinase-induced excision of the gene sequence between the loxP sites (“floxed” gene), which includes an exon sequence, is predicted to produce a truncated AQP2 transcript and no functional protein. Gene targeting was done in ES cells. Homologous recombination was confirmed by Southern blot analysis of ES cell genomic DNA using a 1-kb genomic fragment as a probe, as indicated in Fig. 1A. Apal digestion produced a fragment at 3.2 kb corresponding to the replaced gene containing an Apal site in the Pol2neobpA cassette, and a fragment at 6 kb corresponding to wild-type

Fig. 3. AQP2 immunofluorescence. AQP2 antibody staining of kidney cortex and inner medulla, testis, and vas deferens of tamoxifen-treated WT (left) and AQP2\textsuperscript{flox} (flox; right) mice. Scale bar = 100 μm. Arrowheads indicate retained AQP2 expression in kidney. Arrows indicate AQP2 expression in vas deferens.
gene, indicating correct AQP2 gene targeting with the floxed targeting sequence (AQP2\textsuperscript{flox}). Heterozygous AQP2\textsuperscript{flox} mice were bred with Cre/Esr1 mice (tamoxifen-inducible Cre-recombinase-expressing mice) to generate AQP2\textsuperscript{flox} mice containing multiple Cre/Esr1 expression cassettes.

Homozygous AQP2\textsuperscript{flox} mice carrying the Cre/Esr1 transgene were generated by breeding double heterozygous mice containing AQP2\textsuperscript{flox} and Cre/Esr1. To assess the efficiency of induced Cre-mediated deletion of the loxP-flanked AQP2 gene segment containing exon 2 in the AQP2\textsuperscript{flox} mice, Southern blot analysis was done with genomic DNA from different tissues before and after intraperitoneal injections of tamoxifen over 10 days. After Apal digestion, fragments corresponding to wild-type AQP2, floxed AQP2, and deleted AQP2 genes were predicted at sizes of 6.0, 3.2, and 5.5 kb, respectively. Figure 1A shows a single band at 3.2 kb (in tail DNA) in the homozygous band at 6.0 kb (in kidney DNA) of a wild-type mouse, and a 5.5-kb band and the partial disappearance of 6.0, 3.2, and 5.5 kb, respectively. Figure 1B shows a single band at 6.0 kb (in kidney DNA) of a wild-type mouse, and a single band at 3.2 kb (in tail DNA) in the homozygous AQP2\textsuperscript{flox} mice, as expected. Tamoxifen treatment caused the appearance of a 5.5-kb band and the partial disappearance of the 3.2-kb band, with an efficiency of >90% in kidney and ~80% in liver and testis.

Table 1. Urine and plasma chemistries in wild-type and AQP2\textsuperscript{flox} mice

<table>
<thead>
<tr>
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<th>Wild-type</th>
<th>AQP2\textsuperscript{flox}</th>
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<tr>
<td>Urine output, ml/day</td>
<td>2.2 ± 0.4</td>
<td>25 ± 2*</td>
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<tr>
<td>Urine osmolality, mosmol/kgH\textsubscript{2}O</td>
<td>2,145 ± 198</td>
<td>182 ± 27*</td>
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<tr>
<td>Osmolar excretion, mosmol/day</td>
<td>4,567 ± 395</td>
<td>4,611 ± 297</td>
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<tr>
<td>Creatinine clearance, ml/day</td>
<td>328 ± 36</td>
<td>289 ± 88</td>
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<tr>
<td>Plasma [Cl\textsuperscript{−}], mmol/l</td>
<td>114 ± 1</td>
<td>115 ± 4</td>
</tr>
<tr>
<td>Plasma [K\textsuperscript{+}], mmol/l</td>
<td>8.9 ± 0.3</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>Plasma [Na\textsuperscript{+}], mmol/l</td>
<td>151 ± 1</td>
<td>154 ± 4</td>
</tr>
<tr>
<td>Plasma [urea], mmol/l</td>
<td>9.4 ± 0.9</td>
<td>9.9 ± 1.5</td>
</tr>
<tr>
<td>Plasma [creatinine], mmol/l</td>
<td>0.035 ± 0.01</td>
<td>0.032 ± 0.01</td>
</tr>
<tr>
<td>Urine [Cl\textsuperscript{−}], mmol/l</td>
<td>180 ± 30</td>
<td>12 ± 2*</td>
</tr>
<tr>
<td>Urine [K\textsuperscript{+}], mmol/l</td>
<td>321 ± 26</td>
<td>19 ± 3*</td>
</tr>
<tr>
<td>Urine [Na\textsuperscript{+}], mmol/l</td>
<td>138 ± 39</td>
<td>10.3 ± 0.6*</td>
</tr>
<tr>
<td>Urine [creatinine], mmol/l</td>
<td>1,411 ± 151</td>
<td>117 ± 18*</td>
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<tr>
<td>Urine [creatinine], mmol/l</td>
<td>5.0 ± 0.2</td>
<td>0.34 ± 0.1*</td>
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Values are means ± SE of 3 mice/group. Brackets denote concentration. AQP2, aquaporin-2. *P < 0.01 (Student’s t-test).

Northern blot analysis in Fig. 2A shows the 1.7-kb AQP2 transcript in kidney, inner ear, testis, and vas deferens of wild-type mice, with no detectable transcript in tamoxifen-treated AQP2\textsuperscript{flox} mice. No AQP2 transcript was found in the colon of wild-type or AQP2\textsuperscript{flox} mice. RT-PCR with primers designed to amplify the full-length AQP2 coding sequence gave strong 0.8-kb amplified fragments in cDNAs of kidney, inner ear, testis, and vas deferens of wild-type mice and smaller, weak (0.6 kb) fragments in the tamoxifen-treated AQP2\textsuperscript{flox} mice (Fig. 1B). Very weak fragments at 0.8 kb were seen in the tamoxifen-treated AQP2\textsuperscript{flox} mice when the PCR amplification was carried out for 40 instead of 30 cycles (not shown). Immunoblot analysis showed the expected ~29-kDa band, in kidney, vas deferens, and testis of wild-type mice, which was very faint in the tamoxifen-treated AQP2\textsuperscript{flox} mice (Fig. 1C). A diffuse band migrating at higher molecular size, corresponding to glycosylated AQP2, was seen in kidneys of wild-type mice. No specific band was found in the colon.

AQP2 protein was localized in mouse tissues by immunofluorescence. Figure 3 shows specific AQP2 immunostaining in kidney and vas deferens of wild-type mice (left), with little AQP2 immunostaining in tamoxifen-treated treated AQP2\textsuperscript{flox} mice (right). Residual AQP2 immunostaining in the cortex was less than in the medulla of kidneys of tamoxifen-treated AQP2\textsuperscript{flox} mice. No specific AQP2 staining was detected in testis or in inner ear and colon (not shown).

An analysis of mouse growth by body weight (age 1–5 wk) before tamoxifen administration showed no differences among the genotypes. As expected, the AQP2\textsuperscript{flox} mice had grossly normal appearance, activity, and behavior, and normal renal function.

The urinary concentrating function was compared in wild-type and AQP2\textsuperscript{flox} mice by measuring urine osmolality before and after tamoxifen treatment. There was no significant difference in urine osmolality between wild-type mice (1,984 ± 91 mosmol/kgH\textsubscript{2}O) and AQP2\textsuperscript{flox} mice (1,967 ± 237 mosmol/kgH\textsubscript{2}O) before tamoxifen administration. Urine osmolality in AQP2\textsuperscript{flox} mice decreased remarkably from ~2,000 to <500 mosmol/kgH\textsubscript{2}O by 4–5 days after the first tamoxifen injection, and remained low thereafter at ~200 mosmol/kgH\textsubscript{2}O (Fig. 4A). Daily urinary output in wild-type and AQP2\textsuperscript{flox} mice was measured while they were housed in metabolic cages before and after tamox-
ifen treatment. The AQP2<sub>flox</sub> mice were severely polyuric, excreting ~10-fold greater fluid than litter-matched wild-type mice (Fig. 4B). Urinary concentrating ability was measured in response to an 18-h water deprivation. Urine osmolality in wild-type mice increased from 1,860 to 2,720 mosmol/kgH<sub>2</sub>O, whereas no significant increase was seen in tamoxifen-treated AQP2<sub>flox</sub> mice (Fig. 4C, top). Marked total body weight loss was seen in these AQP2<sub>flox</sub> mice after water deprivation (Fig. 4C, bottom), as expected from their inability to concentrate their urine.

Table 1 summarizes urine and plasma chemistries of wild-type and tamoxifen-treated AQP2<sub>flox</sub> mice at 6 wk after tamoxifen treatment. Plasma concentrations of electrolyte, urea, and creatinine were not significantly different in wild-type and AQP2<sub>flox</sub> mice, nor was creatinine clearance. As expected, daily urine volume was much greater and osmolality lower in the AQP2<sub>flox</sub> mice, and the concentrations of various urinary solutes were reduced.

Despite normal renal function at 6 wk after induction of polyuria, the kidneys of AQP2<sub>flox</sub> mice showed evidence of structural damage from the sustained polyuria. Many kidneys showed evidence of medullary atrophy (Fig. 5A). Histological examination showed dilatation of collecting ducts in the renal cortex and medulla (Fig. 5B). Massively enlarged hydroureters and bladders were seen in the 4OH-TM-treated AQP2<sub>flox</sub> mice (not shown).

Quantitative real-time RT-PCR analysis of kidney homogenates was carried out to examine the effect of induced AQP2 gene deletion on the expression of other renal aquaporins. Figure 6A shows a marked increase in AQP3 transcript but no significant effect on transcripts for AQP1, -4, -6, and -7 in the tamoxifen-treated AQP2<sub>flox</sub> mice. Immunoblot analysis confirmed increased (6 ± 3-fold by quantitative densitometry) AQP3 protein expression in these mice (Fig. 6B). The increased AQP3 protein expression was seen in collecting duct basolateral membranes throughout the cortex and medulla (Fig. 6C).

**DISCUSSION**

We report here the first inducible mouse model of NDI. The motivation for this work was the neonatal mortality seen in several noninducible models of NDI, as reviewed in the introduction. A surviving adult mouse model of NDI has applications for the study of the pathophysiology of NDI in mice and for the evaluation of the in vivo efficacy of NDI therapies such as molecular and chemical chaperones. Our strategy was the introduction of LoxP sites into the mouse AQP2 gene by targeted gene replacement in ES cells and inducible excision of LoxP-flanked gene sequences in adult mice. The LoxP insertions in introns 1 and 2 did not affect AQP2 gene expression or function, as non-tamoxifen-treated homozygous AQP2<sub>flox</sub> mice carrying the Cre/Esr1 transgene had a normal phenotype and

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Fig. 5. Dilatation of collecting ducts after AQP2 gene deletion. A: gross morphology of kidney slices from WT and AQP2<sub>flox</sub> mice at 6 wk after AQP2 gene excision. Scale bar = 2 mm. B: kidney histology in hematoxylin-and eosin-stained sections of renal cortex and medulla. Scale bar = 100 µm.
AQP2 protein expression. After evaluating several Cre-recombinase-expressing mice, including TgN (Mx1-Cre) (Jackson Laboratories), we found adequate collecting duct Cre expression in Cre/Esr1 mice to efficiently excise the LoxP-flanked AQP2 gene sequence and reduce renal AQP2 protein expression in Cre/Esr1 mice to efficiently excise the LoxP-flanked AQP2 gene sequence.

Because the Cre/Esr1 fusion protein is expressed in all cells of homozygous AQP2\textsuperscript{flox} mice, with near complete absence of full-length AQP2 transcript by Northern blot and RT-PCR analysis, and absent AQP2 protein by immunoblot analysis. The efficient renal AQP2 gene excision may be due, in part, to the excellent tamoxifen penetration in collecting duct epithelial cells from both their blood- and urine-facing surfaces.

Tamoxifen-treated AQP2\textsuperscript{flox} adult mice developed a severe urinary concentrating defect with marked polyuria, urinary hyposmolality, and unresponsiveness to water deprivation. This pattern of NDI is substantially more severe and differs from that in other aquaporin knockout mouse models. Mice lacking AQP1 manifest relatively mild polyuria with daily urine output of 5–6 ml, urine osmolality of 500–600 mosmol/kg\textsubscript{H\textsubscript{2}O}, and vasopressin insensitivity (19). The NDI in AQP1 null mice is due to a combination of defective near-isosmolar fluid absorption in the proximal tubule (31) and defective countercurrent multiplication produced by reduced water permeability in thin descending limb of Henle (3) and outer medullary descending vasa recta (27). Mice lacking AQP7, which is expressed only in the S3 segment of the proximal tubule, have little if any defect in urinary concentrating ability (32). AQP3 null mice, which have reduced water permeability in the inner medullary collecting duct, manifest only a mild urinary concentrating defect (18), because in antidiuresis the majority of fluid leaving the ascending limb is absorbed in the cortical collecting duct.

Our previous analysis of AQP2-T126M mutant mice indicated a critical role of AQP2 in neonatal renal function (39). Mice were polyuric and unresponsive to vasopressin and manifested rapidly progressive renal insufficiency with papillary atrophy and death within 6 days after birth. As expected from cell culture experiments (35), AQP1-T126M protein was retained at the endoplasmic reticulum in collecting duct principal cells of the homozygous mutant mice. It was not possible to examine effects of “correctors” of AQP2-T126M cellular processing because of the early neonatal pathology. The inducible AQP2 null mice generated here, when appropriately mated with AQP2-T126M knock-in mice, are predicted to generate conditional knock-in mice suitable for such studies. Adult mice with AQP2-T126M expression would be produced by deletion of the wild-type AQP2 allele in homozygous mice having one inducible wild-type AQP2 allele and one AQP2-T126M mutant allele. Recently, AQP2-F204V mutant mice were identified by forward genetic screening of ethylnitrosourea-mutagenized mice (14). AQP2-F204V is a relatively mild mutation compared with AQP2-T126M (or the null mutant), permitting these mice to survive beyond the neonatal period with a milder form of NDI. When additional mouse models of AQP2 mutation become available, it will be interesting to compare collecting duct water permeability with urinary concentrating function and vasopressin sensitivity.

Studies of renal aquaporin expression indicated significant upregulation of AQP3 transcript and protein in tamoxifen-treated homozygous AQP2\textsuperscript{flox} mice but no differences in the expression of AQP1, AQP4, AQP6, and AQP7. Prior studies

AQP2 protein expression. After evaluating several Cre-recombinase-expressing mice, including TgN (Mx1-Cre) (Jackson Laboratories), we found adequate collecting duct Cre expression in Cre/Esr1 mice to efficiently excise the LoxP-flanked AQP2 gene sequence and reduce renal AQP2 protein expression to near zero.

Because the Cre/Esr1 fusion protein is expressed in all cells of homozygous AQP2\textsuperscript{flox} mice, with near complete absence of full-length AQP2 transcript by Northern blot and RT-PCR analysis, and absent AQP2 protein by immunoblot analysis. The efficient renal AQP2 gene excision may be due, in part, to the excellent tamoxifen penetration in collecting duct epithelial cells from both their blood- and urine-facing surfaces.

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Studies of renal aquaporin expression indicated significant upregulation of AQP3 transcript and protein in tamoxifen-treated homozygous AQP2\textsuperscript{flox} mice but no differences in the expression of AQP1, AQP4, AQP6, and AQP7. Prior studies
have shown increased AQP3 expression by vasopressin (4, 37), although not as high as found here. Interestingly, polyuria significantly reduced AQP3 expression in some rat models, including water-loading (24), bilateral ureteral obstruction (13), hypercalcemia (38), and lithium administration (29). In the present study, tamoxifen-treated AQP2box mice have marked polyuria, likely associated with chronically elevated serum vasopressin concentration and hence AQP3 upregulation.

A secondary purpose of this study was to evaluate extrarenal AQP2 expression. Previous studies have reported AQP2 expression in the rat inner ear (21), colon (9), testis (25), and vas deferens (33). In the rat inner ear, AQP2 expression in the endolympathic sac was found to be increased by vasopressin (30) and proposed to play a important role vestibular function and possibly in the pathogenesis of Meniere’s disease. Immunochemistry in rat testis showed constitutive AQP2 expression in apical membrane of distal vas deferens principal cells (33). The significance of these observations is unclear as NDI subjects with AQP2 mutations have no impairment of vestibular or reproductive functions. AQP2 expression has also been reported in the apical membrane of rat distal colon epithelium (9). Utilizing tamoxifen-treated AQP2box mice as a control, AQP2 transcript expression was found in mouse kidney, inner ear, testes, and vas deferens, but not in colon.

In summary, we have generated and characterized the first inducible mouse model of severe NDI. Unlike prior models of noninducible NDI, adult mice with severe polyuria are able to thrive for more than 6 wk without plasma/urine evidence of renal insufficiency. The strategy of inducible gene deletion should thus be useful in generating mice suitable for testing of therapeutic strategies aimed at correcting defective proteins causing human NDI.

GRANTS

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