Glycogen synthase kinase 3α regulates urine concentrating mechanism in mice

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1Institute of Clinical Medicine, Aarhus University, Aarhus, Denmark; 2Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada; and 3The Kidney Institute, Department of Medicine, University of Kansas Medical Center, Kansas City, Kansas

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Nørregaard R, Tao S, Nilsson L, Woodgett JR, Kakade V, Yu AS, Howard C, Rao R. Glycogen synthase kinase 3α regulates urine concentrating mechanism in mice. Am J Physiol Renal Physiol 308: F650–F660, 2015. First published January 21, 2015; doi:10.1152/ajprenal.00516.2014.—In mammals, glycogen synthase kinase (GSK3) comprises GSK3α and GSK3β isoforms. GSK3β has been shown to play a role in the ability of kidneys to concentrate urine by regulating vasopressin-mediated water permeability of collecting ducts, whereas the role of GSK3α has yet to be discerned. To investigate the role of GSK3α in urine concentration, we compared GSK3α knockout (GSK3αKO) mice with wild-type (WT) littermates. Under normal conditions, GSK3αKO mice had higher water intake and urine output. GSK3αKO mice also showed reduced urine osmolality and aquaporin-2 levels but higher urinary vasopressin. When water deprived, they failed to concentrate their urine to the same level as WT littermates. The addition of 1-desamino-8-D-arginine vasopressin to isolated inner medullary collecting ducts increased the cAMP response in WT mice, but this response was reduced in GSK3αKO mice, suggesting reduced responsiveness to vasopressin. Gene silencing of GSK3α in mpkCCD cells also reduced forskolin-induced aquaporin-2 expression. When treated with LiCl, an isoform nonselective GSK3 inhibitor (41), and proof of concept that inhibition of GSK3 is an essential mechanism by which GSK3α controls urine concentrating capacity.

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GSK3α is important for renal urine concentration. We show here that genetic depletion of GSK3α results in reduced urine concentrating capacity in mice.

MATERIALS AND METHODS

GSK3α KO mouse. The GSK3α KO mouse has been previously described by MacAulay et al. (26). All animal experiments were approved and performed in accordance with guidelines of the University of Kansas Medical Center and Vanderbilt University Medical Center’s Institutional Animal Care and Use Committee.

Metabolic cage experiments. To measure baseline 24-h water and food intake and urine output, mice were housed in metabolic cages (Hatteras Instruments) with free access to food and water. Mice were acclimatized for 3 days. For LiCl treatment experiments, mice were injected with LiCl (4 mmol·kg⁻¹·day⁻¹) by daily intraperitoneal injections. For water deprivation experiments, water bottles were removed for 18 h (from 3:00 PM to 9:00 AM the next day), and spot urine samples were collected before and after water deprivation. Osmolality of urine and plasma was measured by the freezing point depression method using Osmett II (Precision Systems, Natick, MA). Plasma and urine concentrations of Na⁺, creatinine, urea, and plasma Li⁺ were measured using Vitros 950 (Johnson & Johnson) and a BWP XP flame photometer (BWB Technologies).

Immunoblot analysis. Cortical and medullary tissues were homogenized in RIPA buffer and centrifuged at 1,000 g for 15 min at 4°C. Samples were run on 12% polyacrylamide gels (Bio-Rad Protean II). Proteins were transferred to a nitrocellulose membrane (Hybond ECL RPN 3032D, Amersham Pharmacia Biotech) and blocked with 5% nonfat dry milk in PBS with Tween 20 (80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, and 0.1 Tween 20, adjusted to pH 7.4). After being washed in PBS with Tween 20, blots were incubated with primary antibodies overnight at 4°C. The antigen-antibody complex was visualized with horseradish peroxidase-conjugated secondary antibodies using the enhanced chemiluminescence system (Amersham Pharmacia Biotech). Immunolabeling controls were performed using peptide-absorbed antibody.

Immunostaining. Kidneys were cut, fixed overnight in 4% paraformaldehyde at 4°C, washed in PBS, dehydrated in a graded alcohol series, and embedded in paraffin for histological analysis as previously described (11). During normal growth, cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂. After being washed in PBS with Tween 20, slides were mounted with Fluoromount G (SouthernBiotech). 4',6-Diamidino-2-phenylindole was applied to visualize nuclei. Fluorescent images were obtained using a Nikon Eclipse E800 microscope and analyzed using NIS-Elements software (Nikon, USA).

Primary antibodies. Antibodies against GSK3α, GSKβ, cAMP response element-binding protein (CREB), and phosphorylated (p)CREB (Cell Signaling Technologies, Danvers, MA) as well as β-actin (Sigma-Aldrich, St. Louis, MO), AQP-2 (H7661) (31), pSer256-AQP2 (KO407) (3), and Tamm-Horsfall protein (Santa Cruz Biotechnology) were used.

Quantitative real-time PCR. Quantitative PCR on RNA isolated from whole kidney samples was carried out as previously described (36) using an Applied Biosystems ViiA 7 Real-time PCR system. 18S, AQP2, GSK3α, and GSKβ probes were purchased from Applied Biosystems (Foster City, CA). To measure mRNA levels of the vasopressin type 2 receptor (V2R), the following primers were designed: forward 5′-GAGGGAGGAAATGACAGGAG-3′ and reverse 5′-GAGGGAATAGCAACACAGAGG-3′, and β-actin used as a control.

Enzyme immunoassays for cAMP and vasopressin measurements. Urine samples were centrifuged for 5 min at 13,000 rpm and diluted appropriately with enzyme immunoassay buffer. Urinary cAMP, creatinine (Cayan Chemical, Ann Arbor, MI), and vasopressin (Enzo Biochemical, Farmingdale, NY) were measured following the manufacturers’ instructions.

cAMP measurements in acutely isolated inner medullary collecting ducts. Ten-week-old wild-type (WT) or GSK3α KO mice were euthanized, and acutely isolated inner medullary collecting ducts (IMCDs) were obtained essentially as previously described (43). cAMP generation in response to 1 μM dDAVP was measured as previously described with minor changes (36). The tubules obtained were suspended in prewarmed serum-free DMEM-F-12 containing 1 mM 3-isobutyl-1-methylxanthine and incubated at 37°C for 30 min followed by 1 μM dDAVP in a total reaction volume of 0.5 ml and incubated at 37°C for 10 min. The tubule suspension was centrifuged, and the tissue pellet was lysed in 0.1 M HCl, incubated at room temperature for 30 min, and centrifuged at 10,000 rpm for 10 min. The supernatant was directly used for cAMP estimation using a cAMP EIA Kit (Cayan Chemicals) according to the manufacturer’s instruction. Total protein levels were measured in the supernatant, and cAMP levels were expressed per milligram of protein.

Measurement of urine PGE2. Twenty-four-hour urine samples were collected from mice housed in metabolic cages as described above. Urine samples were centrifuged for 5 min at 10,000 rpm, and urinary PGE2 concentrations were measured using the Prostaglandin E₂ EIA Kit (monoclonal) according to the manufacturer’s instructions. Concentrations of PGE₂ are expressed as picograms of PGE₂ per 24-h urine volume per gram body wt.

mpkCCD-C14 cell lines stably transfected with doxycycline-inducible GSK3α short hairpin RNA. Tripz GSK3α short hairpin (sh)RNA plasmid (Thermo Scientific), which encoded a doxycycline-inducible shRNA, and pMD2.g and psPAX2 lentiviral particle packaging plasmids were cotransfected using Lipofectamine 2000 transfection reagent in human embryonic kidney-293 cells according to the manufacturer’s protocol. Lentiviral particles were collected after 48 h, filtered, and stored.

GSK3α shRNA-lentivirus transduction and stable mpkCCD cell line selection were conducted according to Tripz vector manufacturer’s protocols. Mouse collecting duct cells [mpkCCD-C14 (clone 11)] (48) obtained from Dr. Mark Knepper were grown essentially as previously described (11). During normal growth, cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂.

Transduction efficiency after 1 μg/ml doxycycline was evaluated after 24 h by checking fluorescence-TurboRFP expression. Stably transfected cell lines were selected using 2 μg/ml puromycin. To induce GSK3α shRNA and forskolin treatment, mpkCCD-C14 cells stably transfected with GSK3α shRNA were plated on six-well transwell plates. After 4 days, cells were treated with 1 μg/ml doxycycline for 3 days followed by forskolin treatment for 24 h.

Statistical analysis. Values are expressed as means ± SE except for measurements of band density of Western blots, which is expressed as means ± SD. Data were analyzed by two-tailed unpaired t-tests with Welch’s correction and F-test to compare variances. Bonferroni multiple comparisons were used to compare the effect of Li⁺ treatment. Graphpad Prism software was used (version 5.0d). P values of <0.05 were considered significant.

RESULTS

GSK3α is expressed in renal collecting ducts. To determine the expression of GSK3α in the kidney relative to GSK3β, its expression pattern, protein, and mRNA levels were examined. In adult WT mouse kidneys, GSK3α and GSK3β were ubiquitously expressed in renal tubules and could be colocalized
Fig. 1. Glycogen synthase kinase (GSK)3α and GSK3β are expressed in adult mouse collecting ducts. A and B: immunofluorescence staining for GSK3α and GSK3β in wild-type (WT) C57Bl/6J mouse kidneys. A: aquaporin 2 (AQP2; green) and Dolichos biflorus agglutinin (DBA; red) staining represent collecting ducts. B: Tamm-Horsfall glycoprotein (THP; green) staining represents the thick ascending limb (TAL). C: GSK3α and GSK3β mRNA relative to 18S. D: Western blot analysis for GSK3α and GSK3β in isolated renal inner medullary collecting ducts (IMCDs). n = 6. *P < 0.05 compared with the cortex.
Fig. 2. GSK3α knockout (GSK3αKO) mice have polyuria and reduced urine concentrating ability. A: Western blot analysis showing no GSK3α protein in tissue lysates of the medulla and cortex of GSK3αKO mice. GSK3β levels increased in the medulla of GSK3αKO mice compared with WT mice. B: hematoxylin and eosin staining showed no gross morphological abnormalities in GSK3αKO kidneys. C–G: GSK3αKO mice showed increased water intake (C) and urine output (D), reduced urine osmolality (E), and increased urine vasopressin (F) and PGE2 (G) compared with WT mice. n = 8 mice/group. **P < 0.01 and ***P < 0.0001 compared with WT mice.
with AQP2 or Dolichos biflorus agglutinin in collecting ducts in both the medulla and cortex (Fig. 1A). However, we did not see much colocalization of GSK3α or GSK3β with Tamm-Horsfall glycoprotein in the thick ascending limb of the loop of Henle in the outer medulla (Fig. 1B). In the inner renal medulla, GSK3α and GSK3β mRNA levels were not significantly different, but both isoforms were significantly lower in the inner medulla than the cortex (Fig. 1C). GSK3α and GSK3β protein levels were also detected in isolated IMCDs (Fig. 1D). Thus, both isoforms of GSK3 are expressed in the kidney, especially in the renal collecting ducts, an important site for water reabsorption and urine concentration.

**GSK3α KO mice are polyuric under normal conditions.** To determine the role of GSK3α in urine concentration, we examined the effect of its gene deletion in GSK3α KO mice. GSK3α was undetectable in the renal cortex and medulla, whereas GSK3β levels were increased in the medulla of GSK3α KO mice (Fig. 2A). No gross morphological abnormalities, such as cysts or dilated tubules, were detected in GSK3α KO kidneys (Fig. 2B). GSK3α KO mice weighed 13% more than WT mice (Table 1), although the reason for this increase is currently unclear. No significant differences in food intake, plasma osmolality, creatinine, urea levels, or urinary excretion of urea and Na+ were detected among the study groups (Table 1).

Under normal conditions, with free access to food and water, water intake and urine output in GSK3α KO mice were elevated three- and twofold, respectively, and urine osmolality decreased by 25% compared with WT littermates (Fig. 2, C–E). Measurement of AVP levels in the urine has been previously reported as a reliable surrogate for circulating AVP levels (28, 50). In GSK3α KO mice, urinary AVP levels were threefold higher compared with WT mice (Fig. 2F), demonstrating that the urinary concentrating defect in these mice is not due to low vasopressin.

We also measured mRNA levels of the AVP receptor (V2R) and protein levels of urea transporter (UT)-A1, which is threefold higher compared with WT mice (Fig. 2), demonstrating weaker labeling of AQP2 and pAQP2 in medullary collecting ducts of GSK3α KO mice compared with WT mice (Fig. 3D). Importantly, many collecting ducts in the corticomedullary junction of GSK3α KO mice showed no staining for AQP2 (Fig. 3E).

When water deprived for 18 h, medullary AQP2 and pAQP2 protein levels increased significantly in both WT and GSK3α KO mice. The fold increase in AQP2 levels due to water deprivation was not significantly different between WT and GSK3α KO kidneys (1.9- and 1.8-fold, respectively), although pAQP2 levels increased by 2.5-fold in WT kidneys and only 2.1-fold in GSK3α KO kidneys. However, both AQP2 and pAQP2 levels in water-deprived GSK3α KO mice were significantly lower than those in WT mice (Fig. 4, A–C). Consistently, urine osmolality and urinary cAMP levels (16) were lower by 33% and 40%, respectively, in GSK3α KO mice compared with WT mice (Fig. 4, D and E). Likewise, the addition of dDAVP to isolated IMCDs increased the cAMP response in WT mice, but this response was absent in GSK3α KO mice, suggesting a reduced response to AVP in GSK3α KO mice (Fig. 4F).

Taken together, these data suggest that lower cAMP levels and AQP2 expression in GSK3α KO mice could have contributed to their reduced urine concentrating capacity.

**Gene silencing of GSK3α in mpkCCD-C14 cells reduced AQP2 expression.** Previous studies have shown that pharmacological inhibition of GSK3 using LiCl, Zn, and 6-bromoindubrin-3′-oxime can reduce AQP2 levels in in vitro cell culture systems (20, 21, 24). However, these inhibitors are incapable of distinguishing between GSK3α and GSK3β isoforms. To test for isoform-specific roles of GSK3α in the regulation of AQP2, we used gene silencing. A cortical collecting duct cell line, mpkCCD-C14, was stably transfected with doxycycline-inducible shRNA for GSK3α. Polared cells grown on transwells expressed significantly reduced GSK3α protein levels when treated with doxycycline, whereas GSK3β was unchanged (Fig. 5A).

To examine if GSK3α gene silencing can reduce forskolin-induced AQP2 expression, we treated polarized cells with forskolin in the presence or absence of doxycycline. Forskolin is an agonist of adenylyl cyclase and can increase intracellular cAMP levels. Forskolin treatment increased AQP2 in vehicle-treated but not in doxycycline-treated mpkCCD cells (Fig. 5, B and C). We also measured activated CREB (pSer133 CREB) (6). Increased intracellular cAMP and activation of PKA pathway activity can lead to CREB activation. Forskolin treatment increased pCREB levels in vehicle-treated cells but not in doxycycline-treated cells (Fig. 5, B and D). This suggests that cAMP/PKA-mediated pathways are not activated in GSK3α gene-silenced cells to the same extent as normal cells. Immunofluorescence staining revealed that compared with vehicle-treated

### Table 1. Baseline measurements in WT and GSK3α KO mice

<table>
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<tr>
<th>Wild-Type Mice</th>
<th>Glycogen Synthase Kinase 3α Knockout Mice</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>22 ± 1</td>
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<tr>
<td>Food intake, g/24 h</td>
<td>2 ± 0.3</td>
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<tr>
<td>Plasma creatinine, μM</td>
<td>26.5 ± 3</td>
</tr>
<tr>
<td>Plasma urea, mM</td>
<td>11.1 ± 1</td>
</tr>
<tr>
<td>Plasma Na+, mM</td>
<td>155 ± 6</td>
</tr>
<tr>
<td>Plasma osmolality</td>
<td>310 ± 6</td>
</tr>
<tr>
<td>Urinary urea, μmol·min⁻¹·kg⁻¹</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Urinary Na+, μmol·min⁻¹·kg⁻¹</td>
<td>4 ± 0.6</td>
</tr>
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Values are expressed as means ± SE; n = 8–12 mice/group. *P < 0.01.
cells, forskolin-treated cells showed marked increases in labeling of AQP2. Doxycycline-treated cells, on the other hand, showed weaker AQP2 labeling (Fig. 5E). These results suggest that GSK3α can regulate AQP2 expression, and this is consistent with the low AQP2 expression under basal and water-deprived conditions in GSK3αKO mice.

Effect of LiCl treatment on urine concentrating ability in GSK3αKO mice. Although GSK3 inhibition has been implicated to be a major cause for LiCl-induced NDI, it is not clear if Li⁺ causes NDI primarily by inhibiting GSK3 or which GSK3 isoform is a preferential target of Li⁺. Hence, we examined the effect of LiCl treatment on GSK3αKO mice.

Fig. 3. AQP2 abundance is reduced in GSK3αKO mice. Renal medullary AQP2 and phosphorylated (p)AQP2 (pSer256 AQP2) levels were reduced in GSK3αKO mice, as demonstrated by Western blot analysis (A) and quantitation of band density (B). C: AQP2 mRNA relative to β-actin was reduced in GSK3αKO mice. n = 6 mice/group. D: immunostaining showing reduced AQP2 and pAQP2 staining in the renal papilla of GSK3αKO mice. E: in the cortico-medullary junction of GSK3αKO kidneys, many collecting ducts showed very limited AQP2 (green; inset). Collecting ducts with normal AQP2 expression could also be seen in the same area (*). Magnification: ×63. *P < 0.05 compared with WT mice.
LiCl treatment significantly increased water intake and urine output, accompanied by a decrease in urine osmolality in WT mice. In contrast, only small changes were observed in GSK3β/H9251 KO mice (Fig. 6, A–C). For instance, urine output increased by 5-fold on day 6 and 8.6-fold on day 12 of LiCl treatment in WT mice, whereas in GSK3β/H9251 KO mice, urine output was unchanged on day 6 and increased by only 2-fold on day 12 compared with baseline controls (Fig. 6 B). Renal medullary AQP2 levels in WT mice treated with LiCl for 6 or 12 days were significantly lower than baseline controls (Fig. 6, D and F). In GSK3β/H9251 KO mice, however, AQP2 levels did not reduce significantly by 6 or 12 days of LiCl treatment (Fig. 6, E and G). Plasma Li⁺ concentrations were not significantly different in WT and GSK3α/KO mice on day 9 of LiCl treatment (WT mice: 2 ± 0.4 mmol/l vs. GSK3α/KO mice: 1.7 ± 0.6 mmol/l, n = 6), suggesting that deletion of GSK3α does not alter the absorption of Li⁺. Plasma Li⁺ levels were similar to those from an earlier study (37) and in a range that could cause mild toxicity by human standards (45). However, mice did not show any symptoms such as lethargy, loss of body weight, ruffled fur, or diarrhea. Taken together, these data indicate that, in WT mice with intact GSK3α and GSK3β isoforms, LiCl treatment induces NDI, possibly by primarily inhibiting GSK3α. In GSK3α/KO mice, which retain GSK3β and display polyuria at baseline, LiCl treatment does not induce NDI in the short term (6 or 9 days). These results demonstrate that GSK3 plays a major role in Li⁺-induced NDI and suggest that GSK3α could be a primary target of Li⁺ in the initial increase in polyuria and urinary concentrating defect.

**DISCUSSION**

The present study demonstrates, for the first time, that GSK3α is an important regulator of urine concentration by the kidneys and an essential target of LiCl in Li⁺-induced NDI. Gene deletion of GSK3α resulted in reduced AQP2 expression, polyuria, and urine concentrating defect in mice, and gene silencing of GSK3α reduced forskolin-induced AQP2 expression in cultured mPKCCD-C14 cells. A potential contributing
factor to the reduction of AQP2 levels is the impaired cAMP generation in renal collecting ducts. This study also identified GSK3/β as an important target for LiCl in Li+/H11001-induced NDI.

GSK3/β is an important regulator of renal urine concentration. Due to lack of isoform-selective GSK3 inhibitors and the assumption that GSK3β and GSK3α might be redundant in their functions, neither GSK3α expression nor its function in the kidney have been explored. The present study shows that GSK3α is expressed in renal tubular epithelial cells, especially in renal collecting ducts. Gene knockout of GSK3α leads to reduced AQP2, increased water intake, and the production of larger volumes of urine with low osmolality.

Fig. 5. GSK3α gene silencing reduces forskolin (FSK)-induced AQP2 expression in mpkCCD-C14 cells. A: mpkCCD-C14 cells stably transfected with short hairpin (sh)RNA for GSK3α [doxycycline (Dox) inducible] showed significant decreases in GSK3α in cells treated with Dox. B: Western blots showing increases in AQP2 and activated pSer133 cAMP response element-binding protein (CREB) levels after FSK treatment (10 μM for 24 h) in vehicle-treated cells but not in Dox-treated cells. C and D: Quantitation of band density of AQP2 (C) and pCREB/total CREB (D). *P < 0.05; treatment and Western blots repeated for a total of n = 6 study replicates. E: immunofluorescence staining showing more AQP2 expression in FSK-treated cells than vehicle-treated or Dox-treated cells.
Urine concentrating defect could result from low physiological levels of AVP. However, GSK3αKO mice had higher urinary AVP levels and higher water intake (thirst) at baseline. Despite the higher AVP levels, they had lower urine osmolality, reduced AQP2, and were unable to concentrate their urine in response to water deprivation. These findings suggested that the urine-concentrating defect in GSK3αKO mice could be due to a defective renal response to vasopressin.

GSK3αKO mice had lower urinary cAMP levels after water deprivation, suggesting that they could have a reduced response to AVP. The importance of GSK3α in the cAMP-mediated signaling pathway was also supported by in vitro experiments. In mpkCCD cells, gene silencing of GSK3α reduced forskolin-induced pCREB levels. Since the cAMP-PKA-mediated pathway activates CREB (6), the low pCREB levels suggest reduced cAMP-mediated signaling in GSK3α gene-silenced cells. PKA-induced CREB activation is also known to regulate AQP2 expression (15, 27, 47). In a previous study (22) using mpkCCD cells, dDAVP treatment for 24 h increased AQP2 by a PKA- and pCREB-dependent mechanism, although 4 days of dDAVP treatment increased AQP2 by an alternate mechanism. In the present study, forskolin treatment for 24 h increased pCREB and AQP2 levels in normal mpkCCD cells but not in GSK3α gene-silenced cells. This further supports the observation in mice that gene KO of GSK3α can reduce cAMP levels and AQP2 expression.

The observation that baseline urine concentrating ability is disrupted in GSK3αKO mice is in contrast with our earlier observation in mice lacking GSK3β in collecting ducts, which were normal at baseline (36). This raises the possibility that GSK3α, rather than GSK3β, plays a more important role in renal urine concentration and that GSK3α could be able to compensate for the loss of GSK3β.

The possibility that GSK3αKO mice could have altered water or salt transport in other tubular segments of the nephron or have variations in the levels of hormones other than vasopressin cannot yet be discounted. However, we measured PGE2, and our data demonstrated increased urinary PGE2 excretion in GSK3αKO mice. PGE2 can interact with four different EP receptors (EP1–EP4) in collecting ducts (2), and the inhibitory effect of PGE2 on the AVP-induced increase in water permeability is likely to be mediated by EP1 and/or EP3 (12, 44). Thus, one could speculate that PGE2, possibly via EP1 or EP3, might mediate the inhibition of AVP action in the IMCD, leading to decreased cAMP and AQP2 expression in GSK3αKO mice. Nevertheless, our data indicate that GSK3αKO mice have a urinary concentrating defect of renal...
origin with the strong possibility that it is due to a reduced response to vasopressin.

Inhibition of GSK3α is key to the development of Li+-induced NDI. Li+ is a common and effective therapy for bipolar disorders. NDI is the most frequently found undesirable effect of Li+ therapy, and it is found in up to 40% of patients (10, 42). The primary cause of Li+-induced NDI is the decrease in renal AQP2 expression (10), for which several mechanisms have been proposed, including altered AVP/cAMP and cyclooxygenase-2/PGE2 signaling (4, 21, 23, 37).

Multiple studies have demonstrated that inhibition of GSK3β is involved in Li+-induced NDI. This is based on observations that 1) Li+ inhibits GSK3β kinase activity in mouse kidney and cortical collecting duct cells (19–21, 29, 35–37); 2) inhibition of GSK3β by LiCl or GSK3-specific inhibitors can reduce dDAVP-induced AQP2 protein levels in mpkCCD cells (21, 24); 3) inhibition of renal GSK3 coincides with the decrease in AQP2 expression as well as the increase in polyuria in LiCl-treated mice (29, 37); and 4) GSK3β in renal collecting ducts is important for urine concentration (36). However, clear proof that Li+ causes NDI, primarily by inhibiting GSK3, is lacking. Hence, we compared the effect of LiCl on WT and GSK3αKO mice.

The baseline NDI type of phenotype demonstrated by GSK3αKO mice combined with a compensatory increase in vasopressin levels and lower AQP2 are comparable to features of Li+-induced NDI (10, 38, 45), although only to a moderate level. LiCl treatment caused severe NDI and reduced renal AQP2 levels in WT mice within 6 days, consistent with earlier reports on Li+-induced NDI in mice (16, 29, 37). However, in GSK3αKO mice, the effect of LiCl treatment was abolished until 9 days after the start of LiCl treatment, and the onset of mild Li+-induced NDI was evident only by 12 days of treatment. As such, our data suggest that GSK3α could be an important target of LiCl.

Conclusions. The results of the present study demonstrate that GSK3α is important for renal urine concentration and that this isoform may be relatively more important than GSK3β for the regulation of water homeostasis. The present study also revealed that GSK3 inhibition could be a major mechanism for Li+-induced NDI and GSK3α to be a preferential target for Li+. A better understanding of the distinct roles of GSK3α and GSK3β would be beneficial in designing isoform-selective drugs, which could avoid dehydrolation in patients on Li+ therapy.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


