Lithium: a versatile tool for understanding renal physiology

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Submitted 26 December 2012; accepted in final form 7 February 2013

Kishore BK, Ecelbarger CM. Lithium: a versatile tool for understanding renal physiology. Am J Physiol Renal Physiol 304: F1139–F1149, 2013. First published February 13, 2013; doi:10.1152/ajprenal.00718.2012.—By virtue of its unique interactions with kidney cells, lithium became an important research tool in renal physiology and pathophysiology. Investigators have uncovered the intricate relationships of lithium with the vasopressin and aldosterone systems, and the membrane channels or transporters regulated by them. While doing so, their work has also led to 1) questioning the role of adenylyl cyclase activity and prostaglandins in lithium-induced suppression of aquaporin-2 gene transcription; 2) unraveling the role of purinergic signaling in lithium-induced polyuria; and 3) highlighting the importance of the epithelial sodium channel (ENaC) in lithium-induced nephrogenic diabetes insipidus (NDI). Lithium-induced remodeling of the collecting duct has the potential to shed new light on collecting duct remodeling in disease conditions, such as diabetes insipidus. The finding that lithium inhibits glycogen synthase kinase-3β (GSK3β) has opened an avenue for studies on the role of GSK3β in urinary concentration, and GSK isoforms in renal development. Finally, proteomic and metabolomic profiling of the kidney and urine in rats treated with lithium is providing insights into how the kidney adapts its metabolism in conditions such as acquired NDI and the multifactorial nature of lithium-induced NDI. This review provides state-of-the-art knowledge of lithium as a versatile tool for understanding the molecular physiology of the kidney, and a comprehensive view of how this tool is challenging some of our long-standing concepts in renal physiology, often with paradigm shifts, and presenting paradoxical situations in renal pathophysiology. In addition, this review points to future directions in research where lithium can lead the renal community.

arginine vasopressin; aldosterone; purinergic signaling; prostaglandins; glycogen synthase kinase-3; diabetes insipidus

Historical Aspects of Lithium

LITHIUM (FROM THE GREEK WORD LITHOS, meaning “stone”) is a soft, silvery white alkali metal, which does not occur in free form in nature due to its high reactivity. All vertebrate tissues and body fluids contain lithium at low concentrations (106). The primary dietary sources of lithium salts are grains and vegetables, and in some places, drinking water. While there are no specific diseases associated with a dietary deficiency of lithium (cf. potassium or magnesium deficiency), based on the available information and evidence, it was suggested that lithium should be classified as an essential trace element with no specific diseases associated with a dietary deficiency of lithium (cf. potassium or magnesium deficiency). Nutritional and observational studies have suggested some positive role for lithium in human health and longevity (106, 126). Interestingly, two studies, one in the United States and another in Japan, reported that low levels of naturally occurring lithium in drinking water reduced the rate of suicide in the population (30, 79).

In the 19th century, lithium was used as a treatment for gout, since the solubility of uric acid is maximized in the form of lithium urate (22). The use of lithium as an antigout agent was subsequently abandoned because the levels of lithium needed to treat gout proved to be toxic, and the lack of interest on the part of the pharmaceutical industry to manufacture and market a nonpatentable drug. Apart from these medicinal uses, during the late 19th and early 20th century, lithium was added to refreshment beverages to make them more appealing to the public, and as a cure for hangovers. For instance, early versions of both Coca-Cola and 7Up contained lithium. Addition of lithium to beverages in the United States was banned in 1948. Lithium was introduced into modern psychiatric medicine in 1949 by John Cade, an Australian psychiatrist (11, 70), thus heralding the psychopharmacological revolution (31). In 1970, it was approved by the US Food and Drug Administration for the treatment of manic illness, which was later extended in 1974 to the prevention of manic-depressive illness. It is esti-
mated that the use of lithium between 1974 and 1994 has saved $145 billion in hospitalization costs in the United States (49). Recently, the potentially beneficial effects of lithium for the treatment of acute brain injury and chronic neurodegenerative diseases have been discovered and described (see below). This observation made lithium even more interesting to the medical field and thus potentially extends its usefulness in clinical medicine.

Current and Potential Future Clinical Uses of Lithium

One of the main reasons for the survival of lithium for half a century in psychiatric medicine is its ability to counter bipolar disorder (manic-depressive illness). Bipolar disorder is the most serious and prevalent mental illness in the world. In a recent world mental health survey initiated by the National Institute of Mental Health, involving 11 countries across the world, the United States had the highest lifetime and 12-mo prevalence of the bipolar spectrum, 4.4 and 2.8%, respectively (69). The prevalence of bipolar disorder among military veterans is double that in the general population. Posttraumatic stress disorder (PTSD), which has a very high incidence among veterans, predisposes them to depression and substance abuse, leading to bipolar disorder. The recent wars in Afghanistan and Iraq have resulted in a steep increase in PTSD in veterans (41, 110). Currently, ~30% of bipolar patients in the United States receive lithium therapy, where it has distinct advantages over non-lithium-based drugs. First, 25–50% of bipolar patients attempt suicide at least once (44), and lithium is very effective in countering suicidal tendencies in bipolar patients (4, 40, 78). Second, the advances in the pharmacotherapeutics of bipolar disorder over the past 15–20 years have been predominantly in terms of tolerability and safety, with no new treatment(s) being demonstrated to be more effective than lithium (1, 71). Lithium is also used in acute mania, bipolar depression, and as an augmentation agent in the treatment of major depression.

Furthermore, in recent years lithium has emerged as a robust neuroprotective agent for the treatment of acute brain injury (e.g., stroke or ischemia) as well as chronic neurodegenerative diseases, such as Alzheimer’s, Parkinson’s, and Huntington’s (18, 64, 90, 99, 118, 125). Lithium apparently increases cell survival by inducing brain-derived neurotrophic factor and thereby stimulating the activity of antiapoptotic pathways and upregulation of Bcl-2 protein. Consistent with these effects of lithium on neuronal cells, it has been shown by magnetic resonance imaging (MRI) that the grey matter in the brain was increased in the brains of bipolar patients on chronic lithium therapy (72, 103). Currently, lithium is the only known agent to stimulate antiapoptotic activity in the brain. Thus, beyond its current clinical use in bipolar disorder, the neuroprotective ability of lithium implies that it could be used to treat or prevent brain damage following acute injury, or chronic neurodegenerative diseases. Hence, lithium is destined to remain in the market for considerable time into the future.

Insights gained. While lithium has been recognized for decades as a useful therapeutic agent for bipolar disorder, as we begin to better understand the cellular mechanisms underlying its effects, novel targets are coming to light. In other words, because we understand specific pathways that are regulated by lithium, we can envision its use, e.g., as a neuroprotective agent in a variety of conditions in which those pathways are dysregulated.

Lithium’s Effects on the Kidney

One of the organs most adversely affected by lithium treatment is the kidney. Lithium has the potential to lead to markedly impaired urinary concentrating ability that is resistant to arginine vasopressin (AVP), i.e., acquired nephrogenic diabetes insipidus (NDI) (34). Lithium-induced NDI patients present with polyuria, polydipsia, and a markedly reduced ability to concentrate urine, and are unresponsive to AVP (29). In addition, in animal models, lithium causes a marked decrease in the protein abundance of aquaporin-2 (AQP2), the collecting duct water channel (65). NDI occurs in ~40% of lithium-treated patients and is a debilitating condition with considerable morbidity and even mortality, in addition to social inconvenience. The mechanism(s) underlying lithium-associated NDI are not completely understood. The vasopressin resistance of the collecting duct is likely the result of inhibition of glycogen synthase kinase-3, β-isoform (GSK3β), impaired cAMP production, dysregulation of renal prostaglandins, altered purinergic signaling, and changes in renal architecture among other possibilities.

Chronic usage of lithium may result in full-blown nephropathy with reduced glomerular filtration rate (GFR), leading to chronic kidney disease (CKD) (89). While it is estimated that the progression to end-stage renal disease (ESRD) is rare, recent studies have shown modest, yet significant reductions in GFR, as indicated by elevated plasma creatinine, with persistent lithium use (45, 68, 114). Prolonged lithium treatment of humans and rodents may also result in hyperchloremic metabolic acidosis, apparently due to diminished net proton secretion in the collecting duct (renal tubular acidosis) or excessive back-diffusion of acid equivalents, or both (34). These are associated with altered expression of several acid-base transporters in the kidneys of rats with lithium-induced NDI (48). In addition, chronic lithium consumption in humans and rodent models has been associated with potentially irreversible changes to tubular structure (tubular atrophy), and chronic interstitial fibrosis, characterized by increased myofibroblasts, enhanced transforming growth factor (TGF)-β1 expression, and collagen deposition (34, 37, 119). Recently, Walker et al. (119), using cellular and molecular approaches, characterized the chronic interstitial fibrosis in rat kidneys induced by long-term (6 mo) exposure to lithium. Further mechanistic validation of this model may make lithium a very useful tool for the study of the pathogenesis of chronic interstitial fibrosis with minimal impact on renal function.

Lithium is freely filtered at the glomerulus and mostly reabsorbed in the proximal tubule (PT). Thus lithium has been used as a tracer-marker for sodium reabsorption by the PT (7). However, the renal collecting duct appears to be most sensitive to lithium-induced alterations. Lithium is reabsorbed into the collecting duct principal cells by the apical epithelial sodium channel (ENaC) (17). Lithium has been shown to markedly reduce renal expression of a variety of collecting duct principal cell proteins involved in water and salt reabsorption, e.g., AQP2 and ENaC subunits (58, 59). In this vein, amiloride has been shown to be protective against lithium-induced NDI (5, 6, 54). In addition, lithium reduces protein abundances of urea...
transporters UT-A1 and UT-B, thereby reducing medullary interstitial osmolality (53). However, lithium has no discernible effect on proximal tubule and thick ascending limb (TAL) proteins. It should be noted that the effect of lithium on the bumetanide-sensitive Na-K-2Cl cotransporter (BSC1 or NKCC2) is controversial. Lithium increased NKCC2 in one study, but not in another (reviewed in Ref. 101).

A number of recent reviews described the renal effects of lithium (34, 92, 96, 115). Hence, this review does not provide a comprehensive presentation of these effects. Instead, it focuses on how, over the years, lithium has become a versatile tool for understanding the molecular physiology of the kidney and how this tool is changing some of our long-standing concepts in renal physiology, often with paradigm shifts, and revealing paradoxical or puzzling situations in renal pathophysiology.

**Insights gained.** Prescription-dose lithium, although treated by the kidney like sodium, is not benign in that it can cause NDI of varying severity. Moreover, chronic lithium therapy has even been associated with reduced GFR, distal tubular acidosis, and interstitial fibrosis. Lithium mostly influences collecting duct principal cell biology by entering this cell type via the luminal ENaC (sodium channel). Antagonism of the channel, e.g., by amiloride, therefore, may be helpful in reducing lithium reabsorption by the principal cells and thus countering the development of lithium-induced NDI.

**Lithium and AVP**

The neurohypophyseal hormone AVP, through its V2 receptor and the associated cAMP signaling pathway, plays a central role in concentration of urine by the mammalian kidney. The most prominent effects of AVP on the nephron with respect to urine concentration are seen in the TAL and the collecting duct. AVP increases the sodium-absorbing capability of the medullary TAL (mTAL) by stimulating the activity and expression of NKCC2 (BSC1) (21). The net effect is to increase the medullary interstitial tonicity, which is required for rapid absorption of large amounts of water in the adjacent collecting ducts in the outer medulla. AVP increases the osmotic reabsorption of water in the medullary collecting duct by increasing the protein abundance of AQP2 and its apical membrane targeting in the principal cells (reviewed in Ref. 91). Finally, AVP increases the urea recycling through tubular, interstitial, and vascular compartments by the urea transporter (UT) isoforms, thus providing the required osmotic gradients for efficient water reabsorption in the deep inner medulla. AVP increases UT-A2 transcription via a cAMP-responsive element. However, the increase in UT-A1 and UT-A3 expression following chronic AVP infusion appears to be due to an increase in medullary tonicity. The resulting increase in interstitial tonicity may activate UT-A promoter I through its cAMP-responsive element binding protein, leading to increased transcription of UT-A1 and UT-A3. UT-A3 is basically the N-terminal half of UT-A1 (reviewed in Ref. 52).

Lithium-induced NDI is due to the resistance of the kidney to the action of AVP and thus failure of the urinary concentration mechanism. Research conducted over the past two decades identified two transport processes regulated by AVP which are deranged in lithium-induced NDI. Lithium reduces AQP2 protein abundance and thus osmotic water reabsorption in the collecting duct, a process that is not completely recovered even after cessation of lithium treatment (65). Lithium treatment causes reduced UT-A1 and UT-A3 protein abundances in the inner medulla (reviewed in Ref. 52). Discontinuation of lithium-treatment in rodents resulted in restoration of UT-A1 and UT-A3 protein abundances to control levels, despite the lack of restoration of kidney function (52). Amiloride, which ameliorates lithium-induced polyuria, restored the UT-A1 protein abundance (5, 6). Finally, lithium interferes with the AVP-induced phosphorylation of the urea transporters, a cAMP-dependent process, and thus prevents trafficking of the UT transporters to the membrane (52). In contrast, the effect of lithium on NKCC2 expression is less well established (reviewed in Ref. 101). However, treating lithium-induced NDI with a cyclooxygenase (COX)-2 inhibitor improved polyuria via upregulation of AQP2 and NKCC2 (47), which may be due to mechanisms described in the section on Lithium and Prostaglandins (see below). Interestingly, it has been reported that COX inhibition in normal rats enhanced urinary concentrating ability in part through increased expression of the NKCC2 cotransporter in the TAL, which is most likely due to elimination of an EP3 receptor-mediated tonic-inhibitory effect of PGE2 on cAMP production (24). Whether such a phenomenon occurs in normal animals with respect to AQP2 protein abundance, which is also subjected to tonic inhibition by PGE2 in the collecting duct, was not reported.

With respect to the potential cellular and molecular mechanisms of the effects of lithium on the medullary collecting duct, using radioligand binding studies, Hensen et al. (39) reported decreased vasopressin V2 receptor density in the plasma membrane of the renal papilla in lithium-treated rats. In support of this, we found decreased V2 receptor mRNA in the renal inner medulla of lithium-treated mice (130). These observations are consistent with the reported decreased expression of the V2 receptor when blood levels of AVP are high (107, 111), and lithium treatment is associated with increased blood AVP levels. In line with the observation of decreased V2 receptor, we and others found a significant reduction in dDAVP-stimulated cAMP generation in freshly prepared fractions of medullary collecting ducts in lithium-fed vs. regular diet-fed rodents (62, 130). However, the observations made by Li et al. (62) in a mouse cortical collecting duct model (mpkCCDc14) raised questions as to the role of V2 receptor-adenyl cyclase-induced cAMP generation in lithium-induced NDI. They showed that lithium did not affect AQP2 stability, but decreased its mRNA level in these cells. Surprisingly, the effect of lithium was cAMP independent, and it did not alter AQP2-stimulated cAMP production or PKA-dependent phosphorylation of AQP2 or cAMP-responsive element binding protein (62). Further studies are required to elucidate the molecular mechanisms as to how lithium-induced NDI is dissociated from adenyl cyclase activity, and by what mechanisms lithium decreases AQP2 mRNA levels. Such studies should also address how lithium affects cAMP-dependent processes that regulate urea transporter proteins, and why lithium apparently did not have an effect on cAMP-dependent regulation of NKCC2 protein in the TAL. Understanding these mechanisms will identify new targets for the development of therapies for lithium-induced NDI.

**Insights gained.** Decades of studies have led to the theory that lithium interferes with AVP (V2 receptor)/cAMP signal-
ing, leading to reduced expression and trafficking of AQP2, the major apical water channel protein. This appeared to be the major mechanism underlying lithium-induced AVP resistance, leading to NDI. However, recent studies have questioned whether this is the only mechanism or the major mechanism.

**Lithium and Aldosterone**

The effects of lithium on the renin-angiotensin-aldosterone system (RAAS) are not as well defined or understood as those on the vasopressin-collecting duct system. Most, but not all, studies that have measured circulating aldosterone levels find that they are elevated in lithium-treated patients and animal models (76, 77, 113), and may be partly responsible for the hypermagnesemia sometimes observed in treated patients (113). Other studies have suggested that hyperparathyroidism is responsible for hypermagnesemia, as well as hypercalcemia associated with lithium (38, 61).

Even though aldosterone levels are elevated, lithium-induced natriuresis is not always effectively alleviated (77). Similar to that observed for vasopressin, lithium consumption has been associated with aldosterone resistance of the collecting duct (3, 76, 77, 112). In 1973, Baer et al. (3) reported blunted sodium-retaining activity of deoxycorticosterone acetate (DOCA), an aldosterone analog, in rats treated with lithium. Similarly, Thomsen et al. (112), reported that aldosterone or amiloride infusion into lithium-treated rats did not alter sodium excretion, suggesting the aldosterone resistance was due to impaired ENaC activity.

In support of this, more recent studies by Nielsen et al. (75) showed that despite high circulating levels of aldosterone in lithium-treated rats, protein expression and apical labeling of ENaC subunits were significantly reduced. This suggested that decreased expression of ENaC subunits played a role in the “aldosterone resistance.” However, at least in kidney, the β- and γ-subunits of ENaC have been reported to be increased in abundance by vasopressin (20), but not by aldosterone (66); therefore, decreased β- and γ-ENaC on Western blots and stained sections could be attributed to vasopressin resistance. However, α-ENaC has been shown to traffic from intracellular locations into the apical membrane in response to aldosterone (63, 66). When aldosterone was infused into lithium-treated rats, the existing α-ENaC did not traffic normally, suggesting relative aldosterone resistance of the collecting duct (75). In contrast, aldosterone responsiveness of ENaC remained intact in the connecting tubule and late distal convoluted tubule.

To better explore the underlying mechanism(s) for aldosterone resistance of the collecting duct with lithium, Rojek et al. (97) performed a microarray analysis of gene transcripts in the inner medulla from lithium-treated and control rats. An assortment of genes were found to be up- or downregulated, many associated with cellular proliferation. However, they also found an approximate twofold increase in 11β-hydroxysteroid dehydrogenase (type II) expression. This enzyme, expressed primarily in the distal tubule (8), is central in defining the “aldosterone sensitivity” of the post-macula densa. It does so via the degradation of corticosterone/cortisone in these cells, so that aldosterone, which circulates at a much lower level, is able to bind to the mineralocorticoid receptor (MR) and regulate sodium reabsorption. Therefore, it does not seem likely that upregulation of this enzyme would be the cause of aldosterone resistance, unless the upregulation of expression was somehow in compensation for reduced activity. Additional studies are required to fully elucidate these findings. With regard to expression of glucocorticoid receptors (GR) or MR, Semba et al. (105) reported that 14-day lithium treatment in rats increased GR mRNA, but did not affect the expression of MR in rat brain. Other studies (10, 67, 80) have also examined neuronal levels of these receptors in response to lithium and suggest alterations may play a role in some of the protective effects of these drugs. However, additional studies in the kidney will be required to clarify regulation of MR and GR, by lithium in this organ.

Moreover, coadministration of aldosterone to lithium-treated rats worsened the polyuria (76). Since aldosterone has been shown in several studies to facilitate the antidiuretic actions of vasopressin, including increasing cAMP generation (14, 38), these findings were somewhat unexpected. While there were no changes in whole-cell levels of AQP2 between lithium-alone or lithium-plus-aldosterone-treated rats, there was a diminishment in apical and an enhancement of basolateral collecting duct labeling, suggesting lithium had unmasked a role for aldosterone in AQP2 trafficking. One would expect that administered aldosterone might increase the damage to the connecting tubule and late distal convoluted tubule, which apparently retain sensitivity to this mineralocorticoid despite lithium treatment. However, the observed alterations in the AQP2 expression and cellular localization occurred in the collecting duct, which becomes resistant to aldosterone during lithium treatment. Hence, the exact mechanism underlying these findings requires further study.

Upstream of aldosterone, lithium has also been shown to increase plasma ANG II levels (83), likely in response to volume contraction. This should help to alleviate the natriuresis. However, similar to both aldosterone and vasopressin, lithium has been shown to interfere with ANG II, and angiotensin type I receptor (AT1R) signaling. Via these actions, it has been demonstrated to facilitate the protective actions of a RAAS blockade in at least one ischemic model system (124). This is thought to be accomplished by inhibiting inositol monophosphatase (IMP) (102), an enzyme important in the AT1R receptor cascade. IMP inhibition leads to depletion of cellular inositol 1,4,5-trisphosphate (IP₃), a key signaling component in the phospholipase C (PLC) cascade post-AT1R. Xu et al. (124) demonstrated that low-dose lithium combined with captopril prevented stroke and improved survival in salt-loaded, stroke-prone hypertensive rats. They did not observe any increase in plasma lithium levels in the captopril-treated rats.

Nonetheless, some studies have shown increased lithium toxicity when lithium was coadministered with therapies to reduce extracellular fluid volume (ECF), including angiotensin-converting enzyme (ACE) inhibitors, AT1R antagonists, and common diuretics (60). In volume contraction, hyponatremia, ANG II blockade, or distal tubularly targeted diuretics, fractional lithium clearance can be decreased, as lithium is substituted for sodium, leading to increased plasma levels of lithium and renal toxicity (36). For example, thiazides, while quite widespread and effective in controlling blood pressure, often lead to compensatory sodium and lithium reabsorption in the proximal tubule (23). Similar findings have been reported for loop diuretics such as furosemide, as well as ACE inhibi-
tors (13, 36). These interactions reinforce the imperative for clinicians to frequently monitor circulating levels of lithium in high-risk patients, including the elderly due to, in general, lower ECFV, impaired renal function, and hyponatremia.

**Insights gained.** Lithium affects the RAAS and is affected by it. Lithium seems to cause aldosterone resistance in the collecting duct, which may be an important component of the natriuresis that often accompanies the diuresis of lithium. Additionally, interactions between lithium and RAAS-targeted therapies for hypertension or edema are of concern clinically, because volume contraction can result in greater lithium retention.

**Lithium and Prostaglandins**

In both human patients and animal models, lithium-induced polyuria is associated with increased production and urinary excretion of renal prostaglandins. PGE2 is a potent antagonist of AVP action in the collecting duct (35, 98, 127). Hence AVP resistance of lithium-induced polyuria and downregulation of the collecting duct water channel AQP2 have been attributed to increased production of renal PGE2 (95, 108, 120). Administration of indomethacin, which inhibits PGE2 biosynthesis, ameliorated lithium-induced polyuria (2). Studies using inhibitors of COX or mice genetically lacking PGE2-synthesizing machinery also supported the potential involvement of PGE2 in lithium-induced NDI (46, 47).

Despite these well-documented facts, interestingly, recent studies raised questions as to whether PGE2 elevation was coincidental or causative with regard to alterations in AQP2 transcription, while addition of PGE2 alone (without lithium) increased AQP2 protein abundance, mRNA levels, and gene transcription, which opened a window for studies that gene transcription occurs in the collecting duct principal cells. Hence the above mechanisms unraveled by Kortenoeven et al. (55) explain the rationale for the beneficial effects of COX inhibitors as well as ENaC blockers, independent of each other, for the treatment of lithium-induced NDI in the clinic. COX inhibitors, such as indomethacin or celecoxib, apparently prevent PGE2-induced lysosomal degradation of AQP2 and thus restore its protein abundance in lithium-induced NDI (47). ENaC blockers, such as amiloride, decrease the accumulation of lithium in the principal cells and thus counter the reduced AQP2 gene transcription (55). However, there may be other mechanisms, such as altered prostanoid signaling, that may play a role in lithium-induced NDI (see below).

**Insights gained.** PGE2, an antagonist of V2 receptor signaling, and urine concentration has been a useful target for treating lithium-induced NDI in the clinic for a long time. Suppression of biosynthesis of PGE2 by COX inhibitors has been the approach for many years. Recent reports questioned the role of PGE2 in lithium-induced downregulation of AQP2 gene transcription, which opened a window for studies that may provide new therapies for lithium-induced NDI. Such new therapies may not target prostanoid synthesis in the kidney, thus avoid the deleterious effects, such as lithium toxicity.

**Lithium and Purinergic and Prostanoid Signaling**

Although increased expression of COX-1 and COX-2 in lithium-induced NDI has been implicated as the causative factor in the enhanced production of renal PGE2 (56, 95), the availability of free arachidonic acid, the substrate, often limits the amount of PGE2 generated in the cells. The free arachidonic acid pool in the cell represents the net balance of its release from and incorporation into membrane phospholipids. This pool size is very low under basal conditions (9), but during membrane receptor activation the rate of release of arachidonic acid by phospholipases, such as ePLA2, exceeds the rate of its incorporation into cell membranes (27). This results in the accumulation of large quantities of free arachidonic acid in the cell for metabolism by either the COX enzymes or by other routes. Under such circumstances, increased expression of COX enzymes may facilitate increased production of PGE2. In this context, it is important to note that induction of COX-2 expression does not necessarily increase PGE2 production as shown in the lithium-treated mpkCCD cells by Kortenoeven et al. (55) or in dehydrated rats as shown by us (109).

In view of the above, it is apparent that some sort of increased membrane receptor activity needs to take place in lithium-induced NDI to create a large intracellular pool of free arachidonic acid for the synthesis of PGE2 by COX enzymes. One possibility is via increased activity of P2Y receptors that are activated by extracellular ATP/ADP/UTP in an autocrine or paracrine fashion (50, 104, 116). Previously we demonstrated that agonist (ATP/UTP) activation of the P2Y2 receptor in inner medullary collecting duct (IMCD) suspensions induces production of PGE2 and that this mechanism is dependent on COX-1 (121). In addition, we showed that IMCD suspensions...
isolated from lithium-treated rats had enhanced production of PGE_2 in response to a set/clamped level of extracellular nucleotides, such as ATPγS, suggesting that chronic exposure to lithium affected either P2Y receptor expression or post-receptor signaling, leading to enhanced production of PGE_2 (129). Specific agonists (ATP/UTP) to the P2Y_2 receptor, the predominantly expressed P2Y receptor subtype in the IMCD, were among those which provided a greater response in lithium-treated rats. This suggests that increased P2Y_2 receptor activity in lithium-induced NDI has the potential to enhance production of PGE_2, via increased release of free arachidonic acid through a PLC-diacylglycerol-PKC pathway (50). The underlying mechanism for the increase in P2Y_2 receptor signaling activity in lithium-induced NDI is yet to be established.

To understand the role of purinergic signaling, and specifically the P2Y_2 receptor, we have studied the responses to lithium in mice with genetic knockout (KO) of the P2Y_2 receptor (130). We observed that genetic deletion of the P2Y_2 receptor offered significant resistance to lithium-induced NDI as assessed by urine output and osmolality and preservation of AQP2 protein abundance in the renal medulla. However, to our dismay urinary excretion of PGE_2 was not reduced in the KO, relative to WT mice, suggesting that the “protection” at least in these mice was not likely due to reduced PGE_2 synthesis. The observed resistance of KO mice to lithium-induced NDI was not due to consumption of lesser amounts of lithium-added food or a decrease in blood lithium levels. We also had no evidence of reduced lithium accumulation in the inner medulla of KO mice (130). On the other hand, we found that the KO mice had reduced EP3 receptor expression in the IMCD which may have reduced overall PGE_2 signal transmission, perhaps accounting for their protection, despite higher PGE_2 levels.

Accordingly, ex vivo stimulation of medullary collecting ducts from lithium-fed KO and WT mice with PGE_2 generated significantly more cAMP in KO (130%) vs. WT mice (100%), which might account for significantly lesser polyuria and preservation of medullary AQP2 protein in the KO mice (130). Thus antagonism of the P2Y_2 receptor, even if it doesn’t, and perhaps to our benefit, reduce total PGE_2 production, may reduce EP3 receptor levels and boost AQP2 activity and expression. This might avoid some of the side effects of conventional COX inhibitors. Thus our P2Y_2 receptor KO mice provided us with additional clues as to novel targets for the treatment of lithium-induced NDI. Further support for the notion that altered prostanooid receptor signaling can alleviate NDI independently of vasopressin V2 receptor signaling comes from the study by Olesen et al. (82), who showed that vasopressin-independent targeting of AQP2 by selective E-prostanoid receptor agonists alleviates NDI. They showed that in vivo a vasopressin V2 receptor antagonist caused a severe urinary concentration defect in rats, which was greatly alleviated by treatment with butaprost (selective antagonist of the EP2 receptor).

With regard to high urine PGE_2 excretion in lithium-treated P2Y_2 receptor KO mice, it was still possible that increased arachidonic acid to drive its synthesis was derived from the action of a different membrane receptor, even another purinergic receptor such as P2Y_4. In support of this, we did find in our studies with ex vivo preparations of IMCD that ADP also caused a significant increase in PGE_2 release in lithium-fed rats in addition to ATPγS and UTP. ADP did not show such an effect in control rat medullary collecting ducts (129). In parallel, we observed a marked (3.4-fold) increase in the mRNA of the P2Y_4 receptor (responds to UTP, ATP, and ADP) in the inner medulla of lithium- vs. control diet-fed rats (129). Thus these studies support the potential involvement of more than one P2Y receptor subtype in increasing the arachidonic acid pool for PGE_2 production in lithium-induced NDI.

Thus a complex picture of interactions among lithium and prostanooid and purinergic signaling is emerging, which offers novel drug targets for the development of new and perhaps safer therapies for lithium-induced, and hopefully other forms of NDI, such as the one seen in postobstructive uropathy (128). This is very important considering the fact that currently used modalities for the treatment of acquired NDI, such as the combined use of a thiazide with a potassium-sparing diuretic (amiloride) or a prostaglandin synthesis inhibitor, exhibit only varying degrees of success and result in additional side effects (34, 73, 88).

**Insights gained.** Recent studies unraveled the complex interactions between purinergic and prostanooid signaling vis-à-vis lithium treatment in animal models. These studies suggest that instead of suppression of PGE_2, it is possible to alter PGE_2 receptor signaling, resulting in significant amelioration of lithium-induced NDI. Incidentally, these studies also uncovered the potential beneficial effects of targeting purinergic P2Y_2 and P2Y_4 receptors for the treatment of lithium-induced NDI.

**Lithium and GSK3β**

Relatively recently, the mechanism of action for many of lithium’s protective and other effects have been tracked to its identification as an inhibitor of glycogen synthase kinase 3 (GSK3), the ubiquitously expressed serine-threonine kinase. Biochemical, pharmacological, genetic, and rodent behavioral models support the hypothesis that inhibition of GSK3 may represent a target for lithium’s mood-stabilizing properties (32). Conversely, multiple additional classes of mood-stabilizing and antidepressant drugs regulate GSK signaling (32). Recently, GSK3 has been shown to be central in a number of intracellular signaling cascades, including those involved in Wnt, hedgehog, growth factor, cytokine, and G protein-coupled receptor cascades. These pathways are involved in gene transcription, cytoskeletal reorganization, energy metabolism, cell cycle regulation, and apoptosis. During embryonic development, Wnt proteins are required for induction of nephrons in the metanephric kidney. Kuure et al. (57) showed that transient inhibition of GSK3 by two structurally unrelated inhibitors, namely lithium or BIO (6-bromoindirubin-3'-oxime), resulted in differentiation and full segregation of nephron segments in isolated mouse and rat kidney mesenchyme apparently by stabilizing β-catenin. Therefore, GSK3 has become a prominent target for pharmaceutical companies as a treatment for Alzheimer’s disease, diabetes, and cancer.

GSK3 exists as two isoforms (α- and β-) coded by two separate genes with some, but not all, overlapping functions (117, 122). The isoforms are ~98% identical, and lithium effectively inhibits both isoforms. Insulin, like lithium, inhibits GSK3 in a similar manner; thus, in this context, insulin and lithium act similarly to enhance glycogen production which allows for efficient metabolism of cellular glucose and the “antidiabetic” protective effect of lithium or other GSK3 an-
tagonists (28). With regard to Alzheimer’s disease, lithium has been suggested to decrease hyperphosphorylation of tau protein, as well as a number of other transcription factors. In addition, inhibition of the GSK3/Wnt/catenin pathway associated with apoptosis may, in general, be neuroprotective (26).

The action of lithium to inhibit GSK3 may result from competing for a magnesium-binding site within GSK3β (12, 33, 100). Thus higher concentrations of Mg²⁺ may reduce lithium’s potency. Moreover, ATP is a chelator of Mg²⁺ and may reduce its availability, thus affecting the activity of GSK3 in this manner. Lithium may also have more indirect effects in reducing GSK3β activity by upregulating Akt (12).

Due to the pleiotropic actions of GSK3, inhibition of its signaling with lithium is likely to have broad-ranging and very environmentally dependent effects. In terms of cellular microenvironments, GSK3 has been proposed to exist in three pools, with different phosphorylative regulators (123). The first pool seems to be in association with AXIN and likely regulated by LRP 5/6. The second is achieved via phosphoinositide-3-kinase (PI-3K) through Akt phosphorylation at Ser9 or Ser27, and the third pool is associated with Wnt signaling, but may be independent of AXIN.

Both GSK3α and GSK3β are expressed in the kidney (93). With regard to lithium-induced NDI, there is strong evidence that GSK3 plays a critical role in both short-term vasopressin action, and longer term, via collecting duct remodeling. Rao and associates (94) developed a mouse model of collecting duct-specific KO of GSK3β and studied the role of this enzyme in lithium-induced NDI. KO mice were only mildly polyuric in the basal state, but had impaired urine concentrating capacity under water deprivation or treatment with a vasopressin analog. Normal regulation of AQP2 was disrupted, including impaired trafficking, reduced mRNA and protein expression, and reduced phosphorylation of Ser256, a residue shown to be impaired in lithium-induced NDI. KO mice were only mildly polyuric in the basal state, but had impaired urine concentrating capacity under water deprivation or treatment with a vasopressin analog. Normal regulation of AQP2 was disrupted, including impaired trafficking, reduced mRNA and protein expression, and reduced phosphorylation of Ser256, a residue shown to be crucial in AQP2 trafficking. Renal cAMP levels in response to dDAVP or forskolin were lower in KO mice, as well as the activity of renal adenyl cyclase (AC). Similarly, pharmacological inhibition of GSK3 reduced cAMP generation. Overall, these results suggest that GSK3β inactivation or deletion reduces AQP2 expression by modulating AC activity and cAMP generation, thereby impairing responses to vasopressin in the renal collecting duct (94). This may account for the vasopressin-resistant polyuria of lithium-induced NDI. Nonetheless, the mechanism whereby GSK3β regulates AC has not been elucidated; e.g., mRNA or protein levels of any of the isoforms of AC in the GSK3β KO mice have not been reported. Recently, this same group has shown that proximal tubule-specific KO of GSK3β reduces sensitivity to acute renal injury due to HgCl₂ toxicity (42).

Insights gained. GSK3 is an important target of lithium with regard to NDI but is also of interest because it is a kinase central in cellular signaling, and thus can affect a variety of cell processes, e.g., cell growth and metabolism, and of keen interest to researchers in fields such as cancer or diabetes mellitus. Lithium offers a simple tool to inhibit GSK3 and thus for the study of its role in renal physiology and pathophysiology.

Lithium-Induced Remodeling of the Collecting Duct

In the last decade, one of the major basic observations made regarding the renal effects of lithium was that the collecting duct essentially underwent “remodeling” (15, 16, 19). Lithium treatment of rats was shown to increase the proportionate number of intercalated cells and reduce the number of principal cells (based on cell-specific markers) in the inner medulla (15). These changes were found, somewhat counterintuitively, to be associated with a high proliferative rate of principal cells (16). Using cDNA microarray analysis, this same group showed long-term lithium treatment in rats resulted in a decrease in cyclin-dependent kinase inhibitor p27kip1 mRNA and protein, which they speculated might have a role in the altered proliferation (97).

Another study demonstrated that early exposure to lithium can result in permanent remodeling effects on the kidney. In rodents, full maturation of the kidney occurs after birth; thus the effects of lithium on renal development was assessed in pups during the postnatal period (days 7–28) by exposure via mother’s milk (51). Pups from lithium-treated dams had an increased incidence of microcysts in cortical collecting ducts and a reduced volume of cortex and outer and inner stripes of the outer medulla compared with untreated rats. Lithium-treated rats also had increased expression of proliferating cell nuclear antigen (PCNA) in the collecting duct. Many of these alterations were permanent (51). Similarly, lithium-treated patients were shown to have a greater incidence of renal cysts (51).

Many studies implicate inhibition, i.e., serine phosphorylation, of GSK3β in the proliferative and antiapoptotic actions of lithium (25, 93) in the collecting duct. As described in the section above, GSK3β plays a central role in the determination of cell survival as a key component in the growth factor- or insulin-stimulated PI-3-kinase/Akt/mammalian target of rapamycin pathway (84). Increased activity of GSK3β is proapoptotic and preserves differentiation. Other recent studies suggest that the collecting duct may remodel based on functional requirements; e.g., changes in dietary potassium intake have been reported to result in interconversion of principal cells and intercalated cells (85).

Finally, at present it is not known whether collecting duct remodeling is a critical factor in the development of lithium-induced resistance to vasopressin and/or aldosterone. It is also not known whether the observed lithium-induced decreases in V2 receptor density in plasma membranes and/or V2 receptor mRNA in the renal medulla (39, 130) reflect collecting duct remodeling.

Insights gained. In response to lithium, the kidney collecting duct “remodels” primarily as a result of differential proliferative rates of intercalated vs. principal cells. This may be a physiological adaptation to the changing demands on this “tissue,” or, on the other hand, it may be pathological. Additional study of this phenomenon is required for full elucidation. Future studies should also address the contribution of collecting duct remodeling in the development of lithium-induced vasopressin resistance.

Lithium and Proteomics and Metabolomics

Lithium and proteomics. Proteomics is an emerging scientific field that explores the overall expression and composition of intracellular proteins. Since proteins are the ultimate mediators of cellular functions, proteomics, which is a fusion of “protein” and “genome,” is considered as a component of functional genomics. Unlike the study of genomics, the study...
of proteomics is complicated by variation in the expression levels and patterns of individual proteins in the cells of the same organism. However, compared with determination of expression of mRNA, proteomics has certain advantages, because it detects and quantifies the proteins. Proteomic analysis of tissues or cells has several applications in biology and medicine as well as drug development. For instance, proteomic analysis of a group of cells following stimulation by a hormone or agent will provide the extent of influence of that hormone or agent on a variety of cellular proteins, such as structural, signaling, metabolic, and regulatory proteins.

At the laboratory bench, proteomic analysis has three components: a protein-separating technique (e.g., 2-dimensional electrophoresis), a protein analytical technique (e.g., mass spectrometry), and bioinformatics analysis. Using this powerful approach, Nielsen et al. (74) performed proteomic analysis of the IMCD in lithium-induced NDI, which provided insights into the mechanisms for lithium-induced AQP2 downregulation and cellular proliferation. They reported that after 2 wk of treatment with lithium, the IMCD of rats showed altered abundances for 74 proteins compared with control rat IMCD. Bioinformatic analysis of the data indicated that proteins involved in cell death, apoptosis, cell proliferation, and morphology were highly affected by lithium. Members of several signaling pathways were activated by lithium treatment, including PKB/Akt-kinase and MAPK, such as ERK, JNK, and p38. Lithium treatment also increased the intracellular accumulation of β-catenin in association with increased levels of phosphorylated GSK3β (74). Thus this study clearly demonstrated that the cellular effects of lithium treatment are broad and complex, and, as such, a single pathway leading to reduced APQ2 expression and subsequent polyuria is unlikely. However, one cannot rule out the possibility that the observed broad and complex effects may be a conglomeration of primary or direct effects of lithium as well as secondary effects of the original direct effects initiated by lithium.

**Lithium and metabolomics.** Metabolomics represents a snapshot of all the metabolites (intermediate and end products of metabolic pathways) in a cell or tissue at any given time point. It is comparable to the fingerprints left by the metabolic processes, which will give clues about which metabolic pathways are active or not in the cell or tissue under the given conditions.

Modern methods of separation of metabolites and sophisticated detection technology allow us to separate, detect, and identify hundreds of metabolites in any given biological sample at a relatively low cost. Hwang et al. (43) determined the metabolic profile of kidney and urine in rats with lithium-induced NDI by 1H-nuclear magnetic resonance-based metabolomics. They reported decreased levels of organic osmolytes and amino acids in different zones of the kidney and elevated levels of several urinary metabolites which were previously known as biomarkers for kidney cell injury (43). Thus the metabolomics study provided deeper insights into the effects of lithium on the cellular metabolic processes.

**Insights gained.** Proteomic analysis of IMCD during lithium-induced NDI showed that cellular effects of lithium treatment are broad and complex, and as such a single pathway leading to reduced APQ2 expression and subsequent polyuria is unlikely. The metabolomic profiling of kidney and urine during lithium-induced NDI provided insights into the effects of lithium on the cellular metabolic processes. These opened avenues for searching for pathways other than vasopressin-V2 receptor-cAMP signaling for the development of new therapies. Integration of proteomics and metabolomics into functional genomics will hold the key to an understanding of the effects of lithium on the kidney leading to NDI.

**Summary and Future Directions**

Once in a while, a therapeutic drug, by virtue of its unique interactions with kidney cells, becomes an important research tool in uncovering cellular and molecular mechanisms of the organ function and thus significantly enhances our understanding of renal physiology and pathophysiology. In the past, drugs such as aminoglycosides and cisplatin helped us to unravel several aspects of cell biology of the kidney. Similarly, in the past two decades, lithium, a drug in use for more than 50 years for the treatment of bipolar disorder, has become an important tool in unraveling several phenomena in the kidney. Although the precise mechanism(s) by which lithium causes NDI is not known, lithium is leading us to a deeper understanding of renal physiology and pathophysiology, with potential therapeutic implications. More interestingly, understanding the intricate mechanisms of interaction of lithium with kidney cells is changing some of our long-standing concepts in renal physiology, often with paradigm shifts, and presenting paradoxical or puzzling situations. Based on its current therapeutic use in bipolar disorder, as well as its potential future use in treating acute brain injury and chronic neurodegenerative diseases, lithium is expected to stay in clinical use for a long time.

Future studies on lithium should address a number of unresolved issues with respect to the kidney, such as 1) the precise mechanism by which lithium reduces V2 receptor expression and AQP2 protein in the kidney (i.e., AVP resistance); 2) the interaction of aldosterone and AVP in the collecting duct during lithium treatment vis-à-vis collecting duct biology; 3) the cause(s) for increased PGE2 production during lithium-induced NDI; 4) the exact role of PGE2 in the genesis of lithium-induced polyuria; 5) the amelioration of lithium-induced polyuria by means other than suppression of PGE2 biosynthesis; 6) the interaction of purinergic and prostanooid receptor signaling in relation to collecting duct biology in lithium-induced NDI; 7) a deeper understanding of the role of GSK3β in lithium-induced downregulation of AQP2; 8) genetic and cell biological mechanisms of lithium-induced collecting duct remodeling and interstitial fibrosis; 9) the role of collecting duct remodeling in the development of lithium-induced vasopressin resistance; and 10) comprehensive analysis of the cellular metabolic processes induced by lithium, with a view to explore fully how the kidney reacts or responds to lithium. Finally, since lithium therapy for bipolar disorder is a life-long experience in many patients, especially veterans, and currently there are no other drugs to completely and confidently replace lithium in psychiatric medicine, the future studies should also address crucial clinical issues, such as 1) reduction of lithium-induced polyuria by safer methods; 2) reduction of lithium-induced natriuresis and kaliuresis; 3) inhibition of lithium-induced collecting duct remodeling; and 4) prevention or slowing down of lithium-induced interstitial nephritis and nephropathy.
ACKNOWLEDGMENTS

The authors thank Drs. Donald Kohan, Noel G. Carlson, Raoul D. Nelson, Carissa M. Krane, and Lance Miller for excellent collaborations in work presented here.

The use of selective antagonists of purinergic P2Y receptors for the treatment of lithium-induced and other forms of NDI is proprietary to the University of Utah and the Department of Veterans Affairs, is protected by a pending patent (PCT/US/2005/038231), and has been published by the World Intellectual Property Organization (WO/2006/066679).

GRANTS

This work has been supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-061183 and DK81041 (to B. K. Kishore) and DK082507 (to C. M. Ecelbarger), the Department of Veterans Affairs (Merit Review Program), National Kidney Foundation of Utah and Idaho, Western Institute for Biomedical Research, a Catalyst Grant from the University of Utah, and resources and facilities at the Veterans Affairs Salt Lake City Health Care System. C. M. Ecelbarger is an Established Investigator of the American Heart Association.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: B.K.K. and C.M.E. provided conception and design of research; B.K.K. and C.M.E. interpreted results of experiments; B.K.K. and C.M.E. drafted manuscript; B.K.K. and C.M.E. edited and revised manuscript; B.K.K. and C.M.E. approved final version of manuscript.

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