

Dysregulation of Renal Aquaporins and Epithelial Sodium Channel in Lithium-Induced Nephrogenic Diabetes Insipidus

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Summary: Lithium is used commonly to treat bipolar mood disorders. In addition to its primary therapeutic effects in the central nervous system lithium has a number of side effects in the kidney. The side effects include nephrogenic diabetes insipidus with polyuria, mild sodium wasting, and changes in acid/base balance. These functional changes are associated with marked structural changes in collecting duct cell composition and morphology, likely contributing to the functional changes. Over the past few years, investigations of lithium-induced renal changes have provided novel insight into the molecular mechanisms that are responsible for the disturbances in water, sodium, and acid/base metabolism. This includes dysregulation of renal aquaporins, epithelial sodium channel, and acid/base transporters. This review focuses on these issues with the aim to present this in context with clinically relevant features.

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Lithium has been used widely for treating bipolar affective mood disorders in human patients.¹ Lithium treatment, however, is associated with a variety of renal side effects including nephrogenic diabetes insipidus (NDI) (ie, a pronounced vasopressin-resistant polyuria and an inability to concentrate urine²⁻⁴), increased renal sodium excretion, and distal renal tubular acidosis.⁵⁻⁸ Patients who have been treated with lithium manifest a slow recovery of urinary concentrating ability when treatment is discontinued. It is estimated that 1 in 1,000 of the population receive lithium and roughly 20%

to 30% of these patients develop serious side effects including polyuria and renal sodium loss, which to a major extent is attributed to severe down-regulation of aquaporin-2, aquaporin-3, and the epithelial sodium channel ENaC in the kidney collecting duct.^{3,4,9,10}

Moreover, chronic lithium treatment is associated with hyperchloremic metabolic acidosis and distal renal tubular acidosis.⁵⁻⁷ The underlying mechanisms for the impaired urinary acidification in the distal nephron and collecting duct after lithium treatment recently have been, at least partly, identified. The impaired acidification may be the result of the following: (1) an inability to generate a maximum pH gradient across the distal nephron for H⁺ secretion (gradient defect; see Nascimento et al⁶); (2) a primary impairment of the proton pump in the collecting duct (secretory defect; see Halperin et al¹¹); or (3) an unfavorable effect of lithium on the electrical gradient promoting H⁺ secretion (voltage-dependent defect; see Arruda et al¹²). The molecular basis for this, including potential changes in the expression of key renal acid-base transporters, recently was ex-

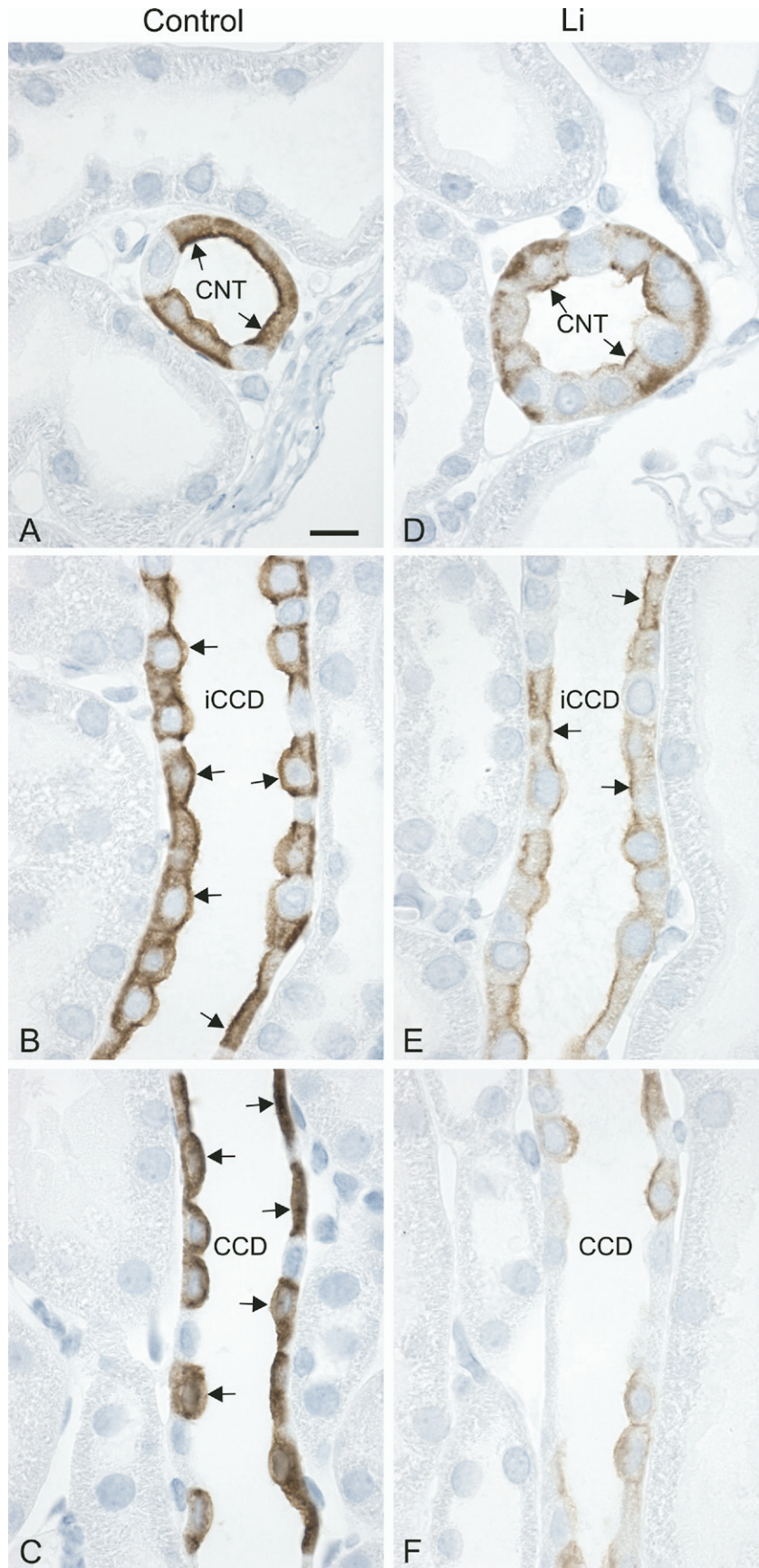
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plored,⁸ and several reports have underscored that there is a major cellular reorganization of the collecting duct with a marked increase in the fraction of intercalated cells and a significant decrease in the fraction of principal cells.^{13,14}

This review focuses on the current understanding on the effects of lithium treatment on kidney function with emphasis on dysregulation of collecting duct aquaporins, epithelial sodium channel ENaC, acid/base transporters, and the marked cellular reorganization of the collecting duct.

RENAL HANDLING OF LITHIUM AND CELLULAR ENTRANCE PATHWAYS

Lithium is excreted almost exclusively by the kidney¹⁵ and is handled similarly to sodium in the renal tubule.^{1,16,17} Approximately 75% of the filtered lithium is reabsorbed in the proximal tubule and in the loop of Henle by substituting for sodium on the Na/H exchanger type (NHE3) and the Na-K-2Cl cotransporter (NKCC2).¹⁶⁻¹⁹ During sodium-replete conditions, renal lithium clearance has been used commonly as an index of tubular fluid delivery from the loop of Henle because no further transport of lithium occurred in the more distal tubule segments.²⁰ However, during sodium depletion there is significant lithium reabsorption from the distal tubule and collecting duct, which can be blocked by the diuretic drug amiloride.²¹⁻²⁵ The effect of amiloride on lithium reabsorption strongly suggests that the amiloride-sensitive epithelial sodium channel ENaC (which has a high permeability for lithium^{26,27}) represents the cellular entry pathway for lithium in the distal tubule segments (ie, distal convoluted tubule, connecting tubule, and collecting duct) where ENaC is expressed. Consistently a recent study has shown that blocking ENaC by amiloride reduces polyuria and increase AQP2 protein expression when administered to rats with estab-

lished lithium-induced NDI.²⁸ Measurements of intracellular [Li⁺] indicate that lithium is actively pumped out of the cells in most tissues.¹⁷ The most likely transporter mediating extrusion of lithium is the basolateral Na/H exchanger type 1.²⁹ The Na-K-ATPase is not thought to play an important role because lithium is an ineffective activator of the pump.³⁰⁻³⁴

Understanding the exact mechanism for entry of lithium into the cells of the distal tubule segments is important because the dominant adverse effects of lithium on regulation of water and sodium handling occur in these segments. Presently, most evidence suggests that ENaC is responsible for cell entry of lithium and the development of the lithium-induced NDI, although definitive studies still are pending.

DYSREGULATION OF AQP2 AND AQP3 REGULATION IN LITHIUM-INDUCED NDI

The progressive polyuria induced by chronic lithium treatment is associated with a parallel down-regulation of both total protein expression and apical trafficking of the vasopressin-regulated AQP2. In lithium-treated rats both AQP2 protein and messenger RNA (mRNA) expression in kidney cortex and medulla progressively were reduced to less than approximately 30% of levels in control rats.^{3,4,13,35} Interestingly, down-regulation of AQP2 protein expression in the kidney cortex was observed mainly in the CCD whereas the connecting tubule was less affected (Fig. 1).³⁶ Consistent with animal studies, a recent *in vitro* study using a highly differentiated mouse clonal CCD principal cell line (mpkCCD_{c14}) showed decreased AQP2 mRNA transcription and down-regulation of AQP2 protein expression whereas AQP2 protein stability was unaffected by lithium.³⁷ Quantitative immunoelectron microscopy of AQP2

Figure 1. Down-regulation of AQP2 in CCD and CNT of lithium-treated rats. Immunoperoxidase labeling of AQP2 in tissue section of kidney cortex of (A-C) control rats and (D-F) rats treated with lithium for 4 weeks. In control rats strong AQP2 labeling was present in the apical plasma membrane domain (arrows) and dispersed within the cytoplasm in (A) CNT, and in the (B) principal cells of the initial CCD (iCCD), and (C) CCD. (F) In the lithium-treated rats the AQP2 labeling in both the apical plasma membrane domain and cytoplasm of the principal cells of the CCD was decreased drastically compared with control rats. The decrease in AQP2 labeling in lithium-treated rats also was observed in the (D) CNT and (E) the initial collecting duct, although the decrease in AQP2 expression was less pronounced. Scale bar, 10 μ m. Used with permission and modified from Nielsen et al.³⁶

labeling in the rat inner medullary collecting duct principal cells showed a marked reduction of AQP2 in the apical plasma membrane as well as in the intracellular vesicles.⁴ The decreased apical expression of AQP2 also is reflected in the decreased urinary AQP2 excretion. AQP2 is excreted in the urine, likely in the form of exosomes originating from multivesicular bodies, and the excretion rate is correlated with vasopressin action and apical AQP2 expression.³⁸⁻⁴¹ Consistently, both experimental animal and human studies with lithium-induced NDI have shown decreased urinary excretion of AQP2.^{41,42} The decreased AQP2 expression, mainly affecting the collecting duct, is likely to play a crucial role in the polyuria, similar to findings in collecting duct-selective AQP2-deficient mice.⁴³

In addition to decreased AQP2 expression, chronic lithium treatment also causes down-regulation of the basolaterally expressed AQP3, as shown by immunoblotting and immunoelectron microscopy.³ AQP3 expression has been shown to be regulated by vasopressin⁴⁴ and changes in tonicity,^{45,46} and plays an important role in water balance. The importance of AQP3 is illustrated by the AQP3 gene-deficient mice that suffer from severe NDI.⁴⁷

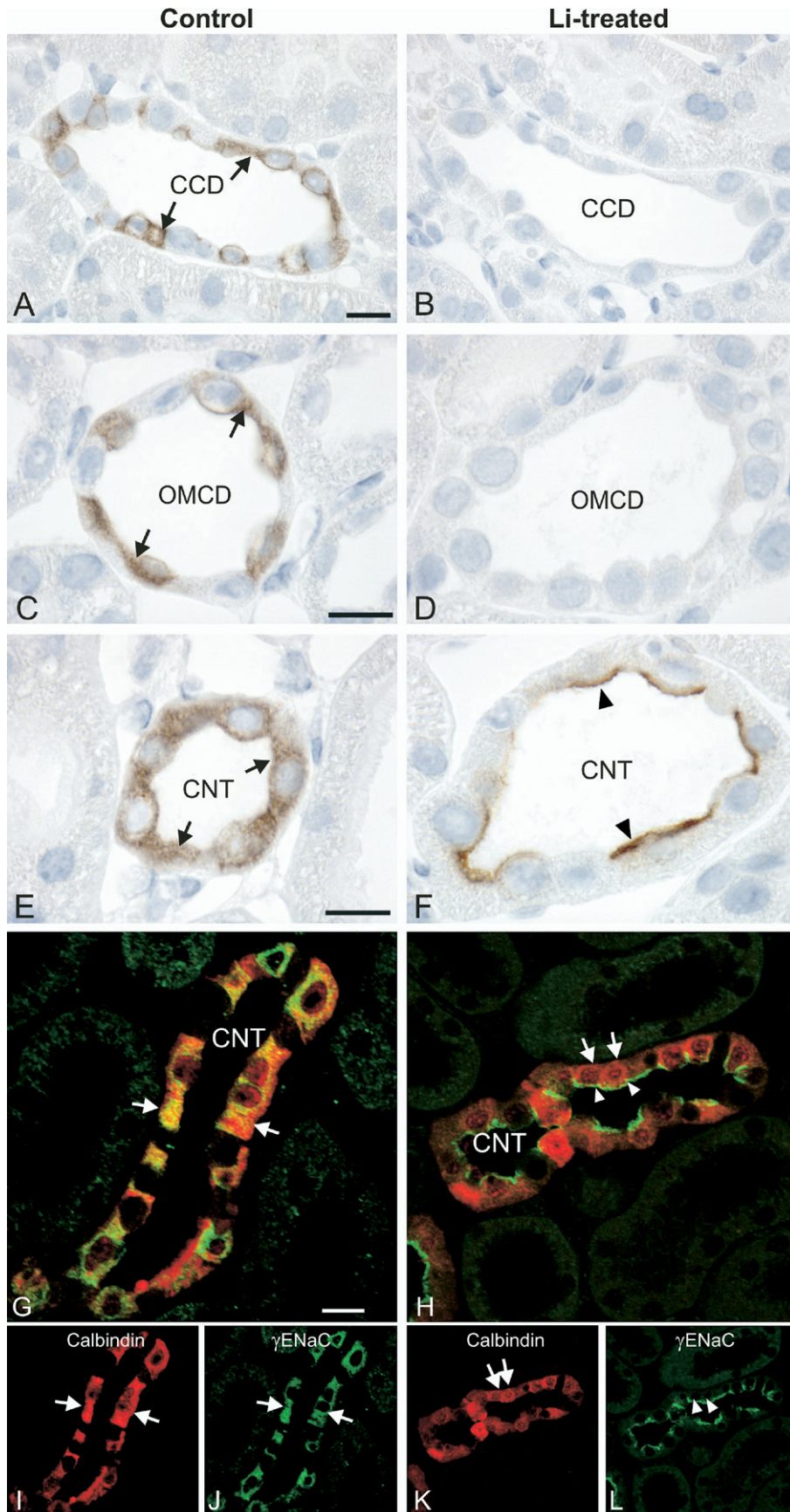
Thus, lithium decreases both protein expression of AQP2 and AQP3 and decreases trafficking of AQP2 to the apical plasma membrane in the collecting duct. These changes are likely to play a major role in the development of lithium-induced polyuria and the urinary concentrating defect.

DYSREGULATION OF RENAL SODIUM HANDLING AND ENaC IN Li-NDI

Chronic lithium treatment increases renal sodium excretion, which is likely to play a role in lithium-induced polyuria and lithium toxicity. In 1949 there were 2 reports of fatal lithium intoxication that suggested that the toxic effects of lithium were increased in patients on a sodium-restricted diet.^{48,49} Later studies in dogs and rats showed a protective effect of high dietary sodium intake on lithium-induced renal sodium wasting.^{15,50-55} As described earlier there is a general consensus among scientists in the field that favors the hypothesis that ENaC represents the entrance pathway for lithium in collecting duct principal cells. Thus, it may be speculated that the plasma concentration of lithium and excretion levels in urine are dependent on ENaC expression and, moreover, that the lithium effect in the collecting duct is proportional to the level of ENaC in the apical plasma membrane. The relationship between sodium content in the diet would be consistent with this because restriction in sodium intake increases aldosterone and hence apical plasma membrane ENaC expression.

Lithium-induced renal sodium wasting was related to a decreased effects of adrenocortical hormones on sodium reabsorption, suggesting that the distal tubule and collecting duct are the targets of lithium.^{15,50,52,54,55} Lithium prevented hypertension in uninephrectomized rats treated with deoxycorticosterone acetate and saline.⁵⁶ Lithium also reduced the amiloride-sensitive so-

Figure 2. Down-regulation of γ ENaC in the collecting duct of lithium-treated rats. Immunoperoxidase and immunofluorescence microscopy of γ ENaC in connecting tubule and collecting duct of control rats and rats treated with lithium for 4 weeks. (A and C) Immunolabeling of γ ENaC is strong and dispersed in the cytoplasm of principal cells of the CCD and outer medullary collecting duct (OMCD) in control rats. (B and D) In the principal cells of the CCD and OMCD of lithium-treated rats the labeling intensity of γ ENaC is strikingly reduced. (E) In the CNT of the control rats γ ENaC was strong and dispersed in the cytoplasm similar to the CCD. (F) Interestingly, expression of γ ENaC in the CNT cells of the lithium-treated rats was not decreased but showed strong labeling of the apical cell domain. The apical targeting of γ ENaC in lithium-treated rats, which received a sodium-restricted diet, was associated with increased plasma aldosterone levels. In lithium-treated rats given extra dietary sodium immunolabeling in the CNT was dispersed mainly in the cytoplasm (not shown). (G-L) To confirm that the tubule segment with apical γ ENaC (green) expression was CNT, immunofluorescence double labeling with the CNT marker protein calbindin-D_{28k} (not marked) was used. (H-L) In the lithium-treated rats tubule segments with apical γ ENaC also were labeled with calbindin-D_{28k} (G-I) whereas control rats showed dispersed cytoplasmic labeling of γ ENaC in the calbindin-positive tubules. The immunolabeling for β ENaC showed an identical labeling pattern (not shown). Arrows indicate dispersed intracellular labeling. Arrowheads indicate apical labeling. Scale bars, 10 μ m. Used with permission and modified from Nielsen et al.¹⁰



dium transport stimulated by aldosterone in chronically catheterized rats with servo-controlled fluid balance, suggesting that decreased ENaC-mediated sodium reabsorption was the cause of lithium-induced renal sodium wasting.⁵⁷ During chronic lithium treatment the sodium wasting is thought to result from altered expression of renal sodium transporters and channels (see later). However, acute lithium administration also decreases tubular sodium reabsorption, which likely is owing to simple competition of lithium and sodium for reabsorption in the renal tubules.⁵⁸ Micropuncture studies in the rat showed that lithium inhibited fractional reabsorption of sodium in the proximal tubule and sodium and water in the distal tubule.^{59,60}

Consistent with the hypothesis that lithium affects ENaC-mediated sodium reabsorption, lithium treatment was shown to cause down-regulation of β ENaC and γ ENaC subunits in the CCD and outer medullary collecting duct (Fig. 2), but not in the connecting tubule cells (CNT).¹⁰ Both mRNA and protein expression of β ENaC and γ ENaC subunits are regulated by vasopressin. Down-regulation of β ENaC and γ ENaC therefore is consistent with decreased vasopressin signaling in the collecting duct principal cells (and the concomitant down-regulation of AQP2).⁶¹⁻⁶³ In addition to the marked effects on β ENaC and γ ENaC protein expression in the collecting duct, lithium treatment in the sodium-restricted rats blocked up-regulation of α ENaC protein expression and trafficking of the ENaC complex from intracellular vesicles to the apical plasma membrane in the collecting duct whereas the connecting tubule was unaffected.¹⁰ The mRNA and protein expression of α ENaC and trafficking of the ENaC complex (with a suggested stoichiometry of 2 α ENaC:1 β ENaC:1 γ ENaC) is known to be induced by dietary sodium restriction and increased plasma aldosterone concentration.⁶⁴⁻⁷⁴ Thus, the absence of α ENaC up-regulation and lack of apical trafficking of ENaC in CCD suggests reduced aldosterone signaling. Consistent with this, aldosterone infusion in rats with lithium-induced NDI neither increased α ENaC protein expression nor apical trafficking of ENaC in CCD (while it occurred in CNT) which may

explain the decreased ability to retain sodium during lithium-induced NDI.⁷⁵

Expression of the α ENaC subunit is thought to be the rate-limiting factor in the assembly and surface expression of the ENaC complex,⁷⁶⁻⁷⁸ although all 3 subunits are required for full channel activity.⁷⁹⁻⁸³ Whether the selective down-regulation of ENaC in the collecting duct is sufficient to cause lithium-induced sodium wasting can be debated. The sodium reabsorption in the collecting duct is smaller relative to the connecting tubule (and distal convoluted tubule). Quantitative analysis based on results from isolated perfused tubules show a 10-fold larger amiloride-sensitive sodium transport capacity in the CNT compared with the CCD.⁸⁴⁻⁸⁷ The relative importance of the CNT over the CCD is underscored further by the very mild phenotype of the CCD-specific α ENaC gene-deficient mice,⁷⁸ compared with the lethal phenotype of mice with the complete lack of either α ENaC, β ENaC, or γ ENaC.^{82,83,88} However, the apparent inability to fully compensate for the sodium wasting associated with the loss of CCD ENaC function that is induced by lithium indicates that the CCD has an important role during severe stress, similar to findings in the CCD-specific α ENaC gene-deficient mice.⁷⁸ Assuming that ENaC is indeed the cellular entry site for lithium, it remains a conundrum why the collecting duct, which during lithium treatment has virtually no apical ENaC, is affected so dramatically by chronic lithium treatment whereas the CNT with normal ENaC expression appears much less unaffected. It certainly indicates that the CCD principal cells are far more sensitive to lithium.

DYSREGULATION OF RENAL SODIUM TRANSPORTERS AND UREA TRANSPORTERS

In addition to the effects of lithium on ENaC, the aldosterone-regulated Na-Cl cotransporter (NCC or thiazide-sensitive cotransporter) expressed apically in the distal convoluted tubule (DCT) also is affected by chronic lithium treatment. In lithium-treated rats on a sodium-restricted diet (causing significantly increased plasma aldosterone) the protein expression of NCC was decreased markedly or unchanged in 2 different studies,^{3,10} suggesting a decreased

response to aldosterone. However, in another study, high-dose infusion of aldosterone increased NCC protein expression in lithium-treated rats on a sodium-replete diet,⁷⁵ suggesting a normal response to aldosterone. NCC is known to be regulated by several other mechanisms⁸⁹ and it remains unclear how lithium affects the regulation of NCC. ENaC (likely entrance site for lithium) is expressed only in the late DCT (DCT2), and not in the early DCT (DCT1). Thus, it may be speculated that lithium only affects the DCT2. Further studies are necessary to explore this.

Other renal sodium transporters upstream from the distal convoluted tubule are unchanged, except the type 2 sodium-phosphate cotransporter (NaPi-2) and electrogenic sodium-bicarbonate cotransporter (NBC1) expressed in the proximal tubule.^{3,8,10}

The NaPi-2 cotransporter contributes to renal proximal tubular phosphate reabsorption.⁹⁰ Inhibition of proximal tubular NaPi2 by parathyroid hormone has been characterized previously by showing a significantly decreased expression of NaPi-2 in the brush border of renal proximal tubules in response to parathyroid hormone treatment.⁹¹ Lithium treatment has been reported to increase serum calcium and lower serum phosphate concentrations and to increase urinary calcium and phosphate excretion in human beings and rats.^{92,93} Because lithium induces hyperparathyroidism, which could result in such electrolyte changes,⁹² decreased expression of renal NaPi-2 could be caused by lithium-induced hyperparathyroidism.

The electrogenic NBC1 localized by functional studies to the basolateral membrane in the proximal tubule contributes to bicarbonate reabsorption and plays an important role in acid-base regulation.⁹⁴ The expression of NBC1 was increased significantly in the kidney cortex of lithium-treated rats.⁸ Several previous studies have shown that systemic pH levels play an important role in the regulation of bicarbonate reabsorption in the proximal tubule.⁹⁵ However, the regulation of NBC1 in the proximal tubule is not well understood and a previous study showed that renal protein expression of NBC1 was not altered significantly in rats with chronic metabolic acidosis induced by oral NH₄Cl loading.⁹⁶ This finding also

is consistent with another study showing unchanged expression of renal cortical NBC1 mRNA in rats with NH₄Cl-induced metabolic acidosis.⁹⁷ One possible explanation for the increased expression of NBC1 in lithium-induced NDI may be that NBC1 could play a role in supporting sodium reabsorption in the proximal tubule in response to the sodium wasting associated with lithium-induced NDI. Chronic lithium treatment is associated with an activated renin-angiotensin-aldosterone system owing to sodium wasting. Because angiotensin II also is known to stimulate the activity of basolateral Na⁺/HCO₃⁻ cotransport in renal proximal tubules,⁹⁸ it is possible that the protein expression of NBC1 could be regulated in response to high angiotensin II levels and contraction of extracellular fluid volume.

In addition to the changes of sodium transporters and ENaC subunits, it recently was shown that the protein expression of urea transport type A1 (UT-A1) located in the inner medulla collecting duct (IMCD) and urea transporter type B (UT-B) located in the descending vasa recta was decreased significantly in the renal inner medulla of rats fed lithium for 10 or 25 days.⁹⁹ The downregulation of UT-A1 and UT-B, therefore, could play a role in the decreased inner medullary interstitial urea concentration in lithium-treated rats² and, thereby, could contribute to the reduction in urine concentrating ability in lithium-treated rats.

MODULATION OF ALDOSTERONE SIGNALING IN LI-NDI AND CENTRAL DI MODELS

It is well established that some diuretics (eg, thiazides) have profound antidiuretic effects in patients with DI including lithium-induced NDI. In 1959, Crawford and Kennedy¹⁰⁰ showed that treatment with chlorothiazide could decrease the urine flow and increase the urine osmolality both in rats and patients with DI. The mechanisms responsible for this paradoxical antidiuretic effect are still only partly understood. However, this lack of knowledge has not been an obstacle to their application in clinical medicine where these drugs have proven valuable in the management of DI, particularly NDI.^{101,102} Consistent with the decrease of urine production by thiazides in lithium-induced NDI it was shown recently that treatment of rats with lithium-induced

NDI with hydrochlorothiazide produced a significant up-regulation of AQP2 in addition to NCC and ENaC.¹⁰³ Furthermore, pharmacologic interference with the renin-angiotensin-aldosterone system also has profound effects in lithium-induced NDI as well as in central DI (see later). There is a complex interaction between vasopressin and aldosterone, and the role of aldosterone in vasopressin regulation of collecting duct water permeability has been the subject of several studies. Mineralocorticoids have synergistic effects on vasopressin-induced osmotic water permeability in toad urinary bladder and isolated perfused CCD from rabbits.¹⁰⁴⁻¹⁰⁶ In normal rats, mineralocorticoids alone have not been shown to affect osmotic water permeability or urine production.¹⁰⁴⁻¹⁰⁸ In contrast, pharmacologic interference with the renin-angiotensin-aldosterone system in polyuric vasopressin-deficient Brattleboro rats using captopril (an angiotensin-converting enzyme inhibitor) or spironolactone (a mineralocorticoid-receptor blocker) caused a marked decrease in urine production.^{109,110} On the other hand, experimental mineralocorticoid deficiency (adrenalectomized and substituted with glucocorticoids) in Brattleboro rats was not associated with altered urine production, although the diluting capacity was decreased when compared with Brattleboro rats with intact adrenal glands.¹¹¹ These initial observations led to a series of further detailed studies that are reviewed briefly later.

The effects of aldosterone and spironolactone treatment on the changes of renal function and renal expression of AQP2 and ENaC in sodium-replete rats with established lithium-induced NDI was examined.^{36,75} Surprisingly, aldosterone treatment caused a dramatic increase in urine production, whereas mineralocorti-

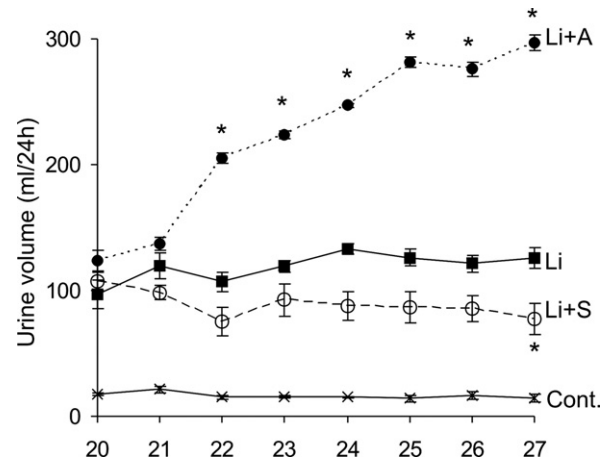
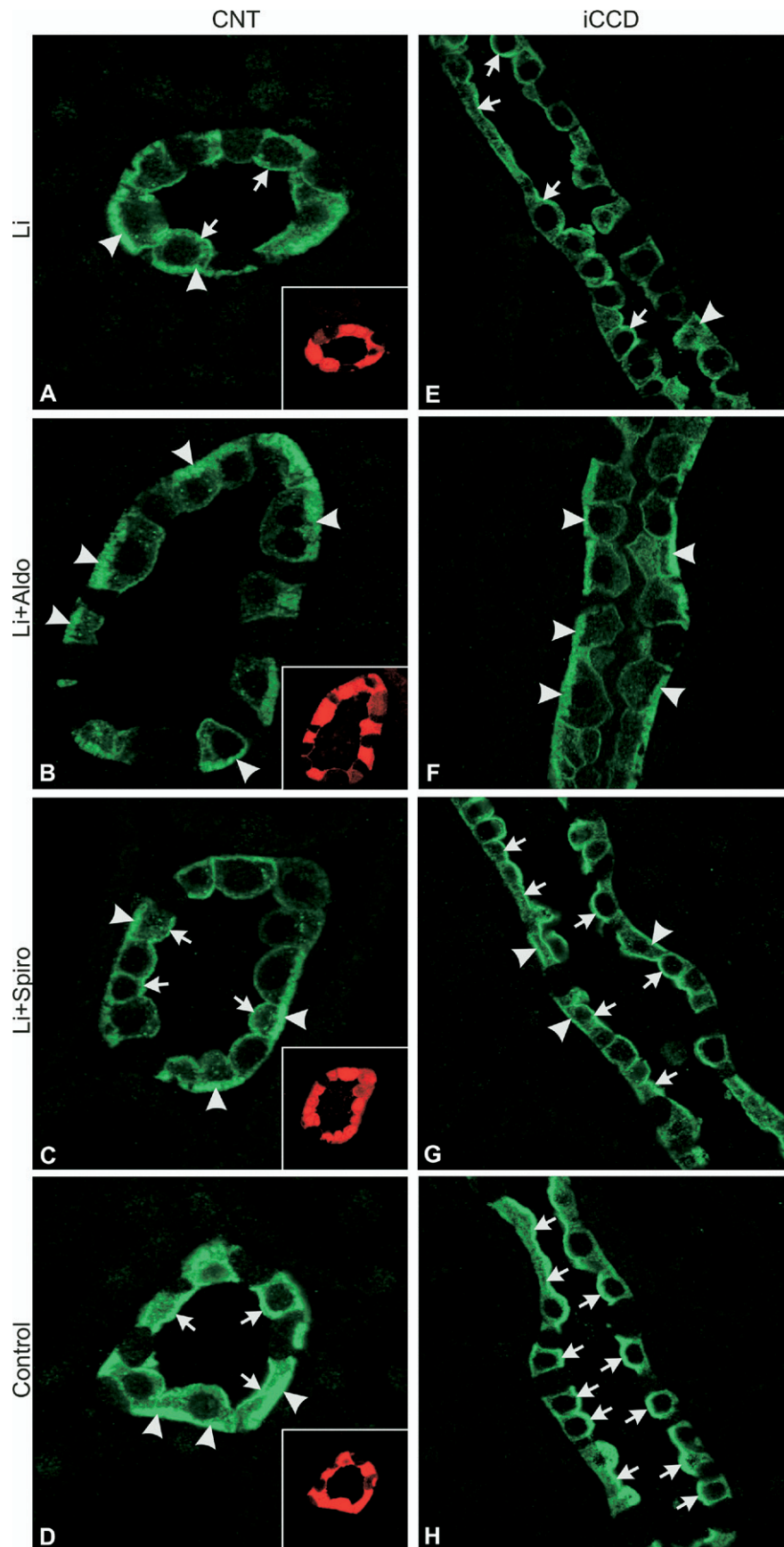


Figure 3. Effect of spironolactone and aldosterone on urine volume in rats with lithium-induced NDI. The aldosterone-treated rats with lithium-induced NDI (Li + A) had progressively increased urinary volume ($*P < 0.05$) compared with rats treated with lithium only (Li). Spironolactone-treated rats with lithium-induced NDI (Li + S) had significantly reduced urinary volume. Untreated rats without NDI (Cont) had approximately 6-fold lower urine volume compared with rats treated only with lithium. Used with permission and modified from Nielsen et al.³⁶

cid-receptor blockage with spironolactone markedly decreased urine production (Fig. 3). Interestingly the increased urine production in aldosterone treatment was associated with a decreased plasma lithium concentration compared with rats treated with lithium alone. The large changes in urine production in response to aldosterone and spironolactone in the rats with lithium-induced NDI were not associated with major changes in the overall protein expression of AQP2. However, aldosterone and spironolactone appeared to have marked effects on the subcellular distribution of AQP2.³⁶ The aldosterone-treated Li-NDI rats had dis-

Figure 4. Increased apical expression of AQP2 in initial CCD in spironolactone-treated rats with lithium-induced NDI. Confocal laser scanning microscopy using immunofluorescence labeling of AQP2 (green) and calbindin_{28k} (red in inset) in the (A-D) CNT and (E-H) initial collecting duct. Tubule segments co-labeled for AQP2 and calbindin-D_{28k} were identified as CNTs. There was a reduced AQP2 labeling in the apical plasma membrane domain in response to aldosterone treatment (Li + Aldo) compared with rats treated with lithium (Li) alone (B versus A). Tubule segments with relatively strong AQP2 labeling but no calbindin-D_{28k} labeling were identified as initial CCD (iCCD) (more distal CCD has very low AQP2 expression as shown in Figure 1). Similar to the CNT, aldosterone caused a decreased labeling in the apical plasma membrane domain (F versus E). Spironolactone treatment (Li + Spiro) resulted in increased apical labeling in iCCD compared with lithium treatment (Li) alone (G versus E). Untreated control rats showed stronger AQP2 labeling in the apical plasma membrane domains, whereas basolateral labeling was unchanged (D and H versus A and E). Arrowheads, labeling in the basolateral plasma membrane domain; arrows, apical plasma membrane domain. Used with permission and modified from Nielsen et al.³⁶



tinctly decreased apical AQP2 expression in the CNT and initial CCD, whereas spironolactone treatment was associated with increased AQP2 labeling in the apical plasma membrane domain in the initial CCD, indicating that the decreased apical AQP2 in the CNT and initial CCD is likely to play a role in the increased urine production in response to aldosterone treatment in rats with Li-NDI (Fig. 4). It is significant to note that aldosterone and spironolactone treatment had similar effects on urine production and changes in the subcellular distribution of AQP2 in vasopressin-deficient polyuric Brattleboro rats, suggesting that the aldosterone-induced change in subcellular distribution of AQP2 and urine production was related to the absence of vasopressin signaling rather than lithium per se.

Additional studies have revealed that combined treatment with dDAVP and candesartan of normal rats led to a blunting of the dDAVP effect with decreased apical AQP2 targeting and increased urine production compared with rats treated with dDAVP alone.¹¹² Similarly, combined treatment with dDAVP and aldosterone blunted the effect of dDAVP with decreased apical (and increased basolateral) AQP2 and increased urine production compared with rats treated with dDAVP alone.¹¹² Thus, several pathways appear to modulate the response to vasopressin and aldosterone with respect to AQP2 regulation both in normal and in polyuric rats (lithium-induced NDI and central DI).

CELLULAR REORGANIZATION OF RENAL COLLECTING DUCT IN Li-NDI

Lithium-induced polyuria is, at least partly, a consequence of reduced expression of AQP2 and AQP3 in the collecting duct principal cells. However, significant changes in the cellular composition or organization of the renal collecting duct has been shown recently in response to lithium treatment of rats and this is likely also to participate. The collecting duct principal cells are the sites for the final regulation of water and sodium reabsorption. Normally, the percentage of principal cells is approximately 60% in the rat CCD, and this number increases slightly along the outer and inner stripes of the outer medullary collecting ducts, reaching approximately 90% in the prox-

imal part of the inner medullary collecting ducts. The remaining cells in the collecting duct are the intercalated cells (ie, 40% in CCD and 10% in proximal IMCD), which are involved in acid-base homeostasis. These cells can be divided further into 3 subgroups: type A intercalated cells, type B intercalated cells, and non-A/non-B intercalated cells. By using different markers for principal cells (anti-AQP2 antibody) and intercalated cells (H^+ -ATPase antibody) on kidney sections the cellular composition of principal cells and intercalated cells was studied in rats treated with lithium for 4, 10, 15 or 28 days. After 10 days of lithium treatment the fraction of principal cells was decreased while the fraction of intercalated cells was increased in IMCD.¹⁴ After 28 days of lithium-treatment the fraction of principal cells in the CCD was decreased to 40% compared to 62% in control rats. In the inner medulla the fraction of principal cells was decreased to 58% compared to 81% in control rats (Fig. 5). The decrease in the fraction of principal cells was followed by an increase in the fraction of intercalated cells.¹³ In parallel with the increased number of intercalated cells the overall protein expression of H^+ -ATPase, which is essential in acid-base balance regulation, also is increased in response to chronic lithium treatment.⁸ Only the type A intercalated cells are increased in number. Remarkably, the changes are completely reversible because the cellular composition of the collecting duct returns back to control levels 4 weeks after cessation of lithium treatment.¹³ Thus, the reduced number of principal cells (together with the reduced AQP2 and AQP3 expression in the remaining principal cells) is likely to participate in the development of polyuria. There are several possible explanations for the lithium-induced remodeling of the collecting duct. One possible explanation could be conversion of principal cells to intercalated cells. However, this is likely not the mechanism because hardly any cells were labeled with both principal cells and intercalated cells cell markers in the time-course studies; double-labeled cells were observed only occasionally. One also could imagine a selective proliferation of intercalated cells but, surprisingly, immunolabeling of the inner medulla

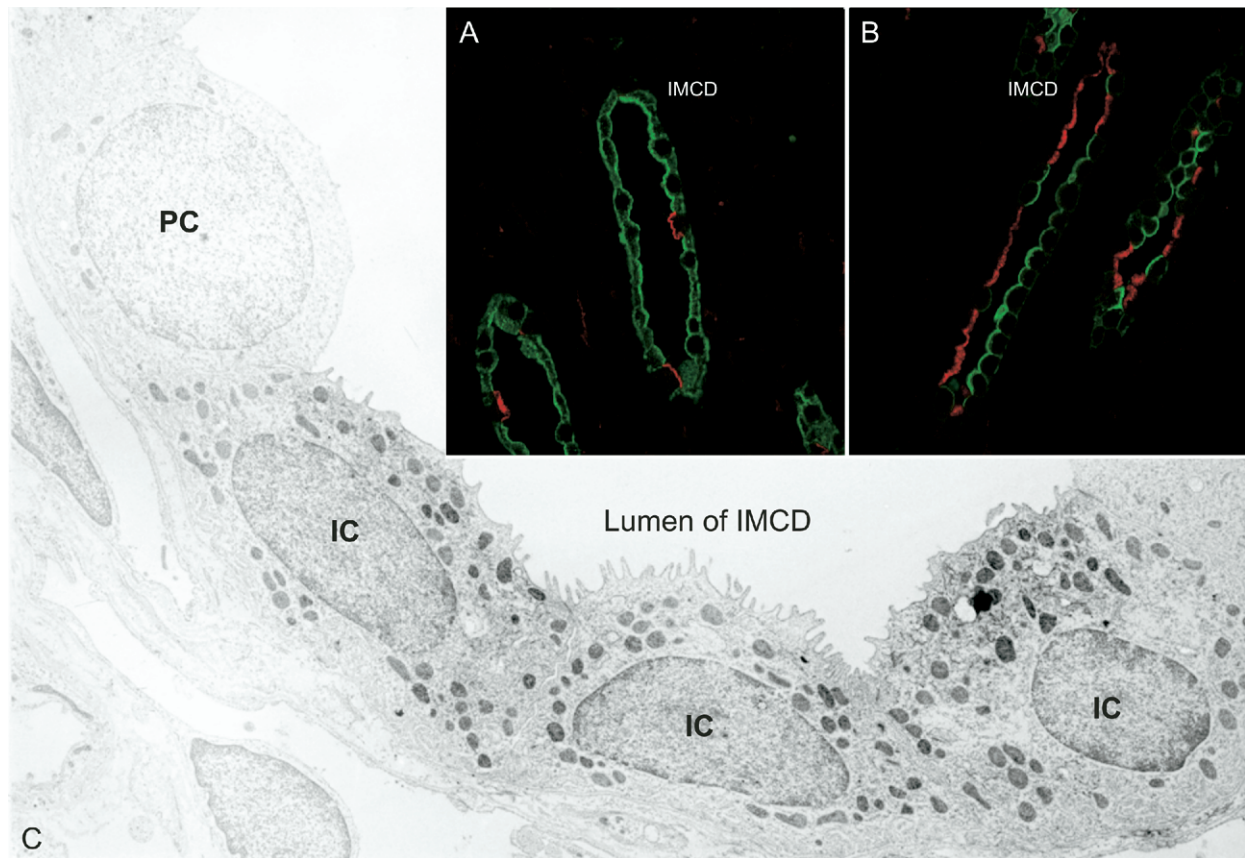


Figure 5. Cellular reorganization and composition of the IMCD. Double labeling of the proximal part of the inner medulla (IM-1) from (A) control rats and (B) lithium-treated rats. Fluorescent double-labeling was performed with polyclonal anti-AQP2 antibody (using a relatively high concentration; green) and monoclonal anti- H^+ -ATPase antibody (red). In both cortex (not shown) and IM-1 an increased density of H^+ -ATPase-positive cells was observed after (B) lithium treatment as compared with (A) controls. Electron microscopy of the inner medulla from lithium-treated rats shows 3 adjacent intercalated cells (IC), which is highly unusual in the normal rat kidney. PC, principal cell. Used with permission and modified from Christensen et al¹³ (A and B) and Kim et al⁸ (C).

with a cell proliferation marker showed a high proliferative rate of principal cells in the inner medulla and a less pronounced proliferation of intercalated cells.¹⁴ It is likely that the massive proliferation of the principal cells leads to a higher turnover of these cells. The principal cells may be removed by apoptosis as suggested by labeling with a marker for apoptosis in cells that did not co-stain with intercalated cell marker. The principal cells also simply may disappear by selective detachment from the basement membrane. Of interest is also the observation that intercalated cells appeared in the middle part of the inner medulla where there are no intercalated cells under normal conditions. Therefore, the increase cannot simply be explained by proliferation of existing

intercalated cells. One explanation could be that the intercalated cells derive from residing or extrarenal stem/progenitor cells. This is supported by the fact that some cells were negative for markers for cell proliferation, intercalated, and principal cells. Thus, further studies are necessary to uncover the underlying mechanisms.

MECHANISM FOR LITHIUM-INDUCED CHANGES IN THE COLLECTING DUCT

As described earlier, it remains likely that the entrance pathway for lithium in the collecting duct principal cell is via ENaC. However, the exact mechanisms leading to the diverse cellular effects including down-regulation of several

proteins involved in water, sodium, and acid-base balance regulation as well as increased proliferation/cell death with collecting duct remodeling are unclear.

The mechanism responsible for the reduction in AQP2 expression is commonly thought to involve interference with the intracellular signaling of vasopressin.^{2,113-115} Under normal circumstances vasopressin activates the vasopressin type 2 receptor and stimulates cyclic adenosine monophosphate (cAMP) production via activation of the G-protein-coupled adenylate cyclase. Increased cAMP causes activation of protein kinase A, leading to increased apical trafficking of AQP2 in the short term whereas the more long-term increase of cAMP leads to AQP2 gene transcription via activation of the cAMP-responsive element binding protein (for review see the article by Nielsen et al¹¹⁶). The reduction in AQP2 expression may be induced by a lithium-dependent impairment in the production of cAMP in collecting duct principal cells,^{2,117,118} indicating that inhibition of cAMP production may in part be responsible for the reduction in AQP2 expression as well as the inhibition of AQP2 targeting to the apical plasma membrane in response to lithium treatment. This is consistent with the presence of a cAMP-responsive element in the 5'-untranslated region of the AQP2 gene^{119,120} and with the finding that mice with inherently low cAMP levels have decreased AQP2 expression (DI +/+ severe mouse).¹²¹

Moreover, it recently has been shown that 24-hour urinary excretion of prostaglandin E₂ (PGE₂) was increased significantly in rats with lithium-induced NDI when compared with control rats.¹²² This is consistent with previous findings showing an increased urinary PGE₂ excretion in lithium-induced NDI in both experimental animals and human patients as well as in patients with congenital forms of NDI.^{123,124} There is also evidence showing that PGE₂ plays a role in modulating the effect of vasopressin on the osmotic water permeability in the renal collecting duct where it attenuates the antidiuretic action of vasopressin; for example: (1) PGE₂ inhibits vasopressin-stimulated cAMP accumulation in the CCD,^{125,126} causing decreased trafficking of AQP2 to the apical plasma membrane^{126,127}; (2) PGE₂ stimulation induces

endocytosis of AQP2 in forskolin-stimulated Madin-Darby canine kidney (MDCK) cells, suggesting that PGE₂ induces internalization of AQP2 independently of AQP2 dephosphorylation¹²⁷; and (3) the diuretic effect of PGE₂ also includes the cAMP and Ca²⁺-independent activation of the Rho-kinase and formation of F-actin.¹²⁸ Furthermore, acute infusion of PGE₂ into normal animals has been shown to cause water diuresis¹²⁹ and to inhibit water absorption in collecting ducts.¹²⁵ PGE₂, therefore, appears to antagonize the hydroosmotic effect of vasopressin and thus PGE₂ may counteract vasopressin action in lithium-induced NDI, serving as a local feedback regulator of the antidiuretic action of vasopressin. Consistent with this, a recent study showed that cyclooxygenase 2 (COX-2) inhibition prevented lithium-induced polyuria and an increased urinary PGE₂ excretion in COX-1 null mice (COX-1 -/- mice), suggesting that COX-2 and/or COX-1-induced PGE₂ production could, at least in part, play a role in lithium-induced polyuria.¹³⁰

In addition to the studies focusing on the effects of lithium on the classic vasopressin signaling cascade, other studies have indicated a potential role of non-vasopressin-mediated effects including the setting of lithium-induced NDI (see Marples et al⁴ and Umenishi et al¹³¹).

PROTEOMIC ANALYSES: UNDERLYING MECHANISMS OF LITHIUM-INDUCED CHANGES

Given the significant effects of lithium, combined proteomics and pathway analysis studies were undertaken to identify novel candidate proteins and signaling mechanisms affected by lithium (see Nielsen et al¹³² for further details). By using differential 2-dimensional gel electrophoresis combined with mass-spectrometry we identified 6 and 74 proteins with altered abundance compared with controls after 1 and 2 weeks of lithium treatment, respectively. Bioinformatics analysis of the data indicated that proteins involved in cell death, apoptosis, cell proliferation, and morphology are highly affected by lithium. Follow-up studies revealed that several signaling pathways including the protein kinase B (PKB, also known as Akt) and the mitogen-activated protein kinases (MAPKs) in-

cluding the extracellular signal-regulated kinase (ERK), the Jun N-terminal kinase (JNK) and p38 MAPK were activated by lithium-treatment. Activated PKB, via the apoptosis signal-regulation kinase 1 (ASK1), is a potential mediator of both JNK and p38 signaling pathways. In addition, p38 is centrally involved in apoptosis and cytoskeleton reorganization after cell stress via its interaction with heat shock protein 27. Stimulation of the ERK signaling cascade modulates numerous cellular functions, including cellular proliferation, differentiation, and survival. Interestingly, ERK inhibitors have been shown to block AVP-induced increases in AQP2 expression.¹³¹ Thus, increased ERK activation after lithium treatment may be a cellular response to limit down-regulation of AQP2.

The effects of various signaling cascades also suggested that central components of the Wnt/ β -catenin pathway involved in proliferation, differentiation, and apoptosis could be affected. One central component of this pathway is the lithium sensitive glycogen synthase kinase type 3 β .^{133,134} Indeed lithium treatment was associated with increased phosphorylation of GSK3 β consistent with previous studies.¹³⁰ GSK3 β functions as a negative regulator of the Wnt/ β -catenin pathway by phosphorylating β -catenin, and overexpression of GSK3 β has been shown to make cells more sensitive to pro-apoptotic stimuli.¹³⁵ Interestingly, inhibition of GSK3 β by lithium results in an increase in local PGE2 excretion,¹³⁰ which may counteract AVP actions by causing endocytic retrieval of AQP2, thus impairing urinary concentrating ability.¹²⁷

Another component of the Wnt/ β -catenin pathway is β -catenin, which regulates cell to cell adhesion by interacting with cadherin in addition to serving an activator of T-cell factor (TCF) dependent transcription factor during conditions with decreased GSK3 β activity (as with lithium treatment).¹³⁶ Immunohistochemistry showed that lithium induced an intracellular accumulation of β -catenin,¹³² which in turn regulates transcription of target genes. Interestingly, mouse and rat AQP2 gene 5' flanking regions contain the TCF consensus sites involved in mediating β -catenin-regulated gene transcription. Thus, lithium may cause down-

regulation of AQP2 via a transcriptional mechanism, which also recently was suggested.³⁷ Furthermore, TCF-dependent transcription has been shown to regulate a number of proteins involved in cell-cycle entry and also may play a role in the principal cell proliferation observed with lithium treatment.

CONCLUSIONS

Lithium-induced NDI is a frequent side effect of the commonly used lithium treatment of bipolar disorders. By using so-called antibody-based targeted proteomics, regulation of selected renal transporters and channels has been examined to establish the integrated response to lithium-induced NDI. Lithium-induced NDI results in severe down-regulation of AQP2, AQP3, and the epithelial sodium channel ENaC in the kidney collecting duct, which is associated with marked polyuria and modest sodium loss. Recent studies have shown that interference with the renin-angiotensin-aldosterone system dramatically affected urine production. Spironolactone treatment markedly decreased urine production in rats with lithium-induced NDI and was associated with increased apical AQP2 expression in the initial CCD principal cells. Conversely, aldosterone treatment of rats with lithium-induced NDI decreased apical AQP2 expression in the CNT and initial CCD and produced an increase in urine output. Moreover, lithium-induced NDI causes a major cellular reorganization of the collecting duct with an increase in the fraction of intercalated cells and a significant decrease in the fraction of principal cells. Novel proteomic analyses have provided insights into potential mechanisms that are involved in mediating the effects of lithium. These recent studies may open a new treatment avenue in clinical NDI cases. Thus, future clinical studies should address whether mineralocorticoid-receptor antagonists (and possibly amiloride) would have additional beneficial effects to the already existing treatment with non-steroidal anti-inflammatory drugs and thiazides in patients with NDI.

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