Lithium-induced Nephrogenic Diabetes Insipidus: Renal Effects of Amiloride

Jennifer J. Bedford,* Susan Weggery,* Gaye Ellis,* Fiona J. McDonald,† Peter R. Joyce,‡ John P. Leader,* and Robert J. Walker*

Departments of *Medical and Surgical Sciences and †Physiology, University of Otago, Dunedin, New Zealand; and ‡Department of Psychological Medicine, University of Otago, Christchurch, New Zealand

Background and objectives: Polyuria, polydipsia, and nephrogenic diabetes insipidus have been associated with use of psychotropic medications, especially lithium.

Design, setting, participants, & measurements: The impact of psychotropic medications on urinary concentrating ability and urinary aquaporin 2 (AQP2) excretion was investigated after overnight fluid deprivation, and over 6 h after 40 μg of desmopressin (dDAVP), in patients on lithium (n = 45), compared with those on alternate psychotropic medications (n = 42).

Results: Those not on lithium demonstrated normal urinary concentrating ability (958 ± 51 mOsm/kg) and increased urinary excretion of AQP2 (98 ± 21 fmol/μmol creatinine) and cAMP (410 ± 15 pmol/μmol creatinine). Participants taking lithium were divided into tertiles according to urinary concentrating ability: normal, >750 mOsm/kg; partial nephrogenic diabetes insipidus (NDI), 750 to 300 mOsm/kg; full NDI, <300 mOsm/kg. Urinary AQP2 concentrations were 70.9 ± 13.6 fmol/μmol creatinine (normal), 76.5 ± 10.4 fmol/μmol creatinine (partial NDI), and 27.3 fmol/μmol creatinine (full NDI). Impaired urinary concentrating ability and reduced urinary AQP2, cAMP excretion correlated with duration of lithium therapy. Other psychotropic agents did not impair urinary concentrating ability. Eleven patients on lithium were enrolled in a randomized placebo-controlled crossover trial investigating the actions of amiloride (10 mg daily for 6 wk) on dDAVP-stimulated urinary concentrating ability and AQP2 excretion. Amiloride increased maximal urinary osmolality and AQP2 excretion.

Conclusions: By inference, amiloride-induced reduction of lithium uptake in the principal cells of the collecting duct improves responsiveness to AVP-stimulated translocation of AQP2 to the apical membrane of the principal cells.

Lithium is a common therapeutic agent used to treat patients with various mood disorders. However, it has been associated with several forms of renal injury (1), the most prevalent of which is impaired urinary concentrating ability, which is estimated to be present in at least 50% individuals on chronic lithium therapy. Initially, the decreased urinary concentrating ability is largely reversible after cessation of lithium. However, with continued treatment, this defect translates into overt and irreversible polyuria and polydipsia in up to 20% of unselected cases (2–4), which is resistant to the actions of arginine vasopressin (AVP) (nephrogenic diabetes insipidus [NDI]). This functional lesion is associated with a chronic focal interstitial fibrosis predominantly in the medullary region of the kidney (5–7), which may be progressive, leading to end-stage renal failure (7). It has been proposed that the centrally driven polydipsia and polyuria, which accompany many psychiatric disorders, may contribute directly to the renal injury, rather than lithium alone. Movig et al. have suggested that the concomitant use of serotonergic antidepressants with lithium is associated with a higher incidence of polyuria (8).

Lithium enters the collecting duct principal cells predominantly via the epithelial sodium channel (ENaC) (9) located on their apical membranes. ENaC shows high selectivity for both Na⁺ and Li⁺, is upregulated by aldosterone, and is inhibited by low concentrations of amiloride (10). A number of different mechanisms may be involved in lithium-induced changes in tubular cell water permeability. Briefly, lithium inhibits AVP-stimulated translocation of cytoplasmic urinary aquaporin 2 (AQP2) to the apical membrane via inactivation of adenyl cyclase and subsequent inhibition of protein kinase A-induced phosphorylation of cytoplasmic AQP2, a step that is essential for its transport to, and insertion into, the membrane (11). Failure of AQP2 insertion leads to delivery of a hypo-osmotic fluid to the medullary collecting duct, whose capacity to reabsorb water is thereby limited (12,13), resulting in the excretion of large volumes of dilute urine. Long-term exposure to lithium may also down-regulate AQP2 gene expression (14).

Baumgarten et al. demonstrated that a reduction in urinary AQP2 excretion may be a useful marker of urinary concentrating defects (15), and we have confirmed the correlation of urinary AQP2 measurements and concentrations in response to water deprivation and desmopressin (dDAVP) administration in normals (16) and healthy participants taking lithium (250 mg/d) for 4 wk (17).
Although there have been a number of experimental studies investigating the actions of lithium, there are currently no clinical data correlating lithium exposure with changes in urinary concentrating ability, responsiveness to dDAVP, and urinary AQP2 excretion. Amiloride has been known to inhibit the uptake of lithium in the collecting duct and has been used clinically to ameliorate polydipsia, psychotropic medications, AVP-stimulated urinary concentrating ability, and urinary AQP2 excretion; and 2) a randomized double-blind, placebo-controlled, crossover study of the effects of amiloride on AVP-stimulated urinary concentrating ability and urinary aquaporin and cAMP excretion in individuals with mood disorders treated with lithium.

Materials and Methods

Participants

In the first study, 45 participants (26 females, 19 males) on lithium therapy for the management of their mood disorder were matched with 42 participants (36 females, 6 males) receiving therapy for a mood disorder but who had not been exposed to lithium therapy and were managed with alternative psychotropic agents. Inclusion criteria included individuals with bipolar disorder who were clinically stable with no change in their medications over the preceding 3 mo and no known history of renal disease. Exclusion criteria included the inability to give informed consent, a history of known renal disease, the use of a diuretic or angiotensin converting enzyme inhibitor, inability to comply with an overnight water restriction, unstable psychiatric condition, or recent changes in psychotropic medications. Participants' demographic data are presented in Table 1. Plasma lithium concentrations were within the accepted therapeutic range (0.5 to 1.0 mmol/L) (Table 2). All participants gave written informed consent to take part in the study, which was approved by the Otago and Canterbury Regional Ethics Committees.

For the second study, individuals from the cross-sectional study on lithium therapy were approached to take part in a randomized, double-blind crossover study to investigate the effects of amiloride on the urinary concentrating ability. Eleven subjects from the above cohort (9 females, 2 males) consented to the study. The trial is registered on the Cochrane Renal database (www.cochrane-renal.org) (CRG060500004) (a randomized double-blind, placebo-controlled crossover study of the effect of amiloride over 6 wk, on renal water handling in individuals with a bipolar or unipolar disorder, requiring lithium therapy).

Participants' demographic data are presented in Table 3, and baseline physiologic data are given in Table 4. All participants gave written informed consent to take part in the study, which was approved by the Otago Regional Ethics Committee. Randomization was by a computer-generated code.

Following randomization, participants received either placebo or amiloride for 6 wk, followed by a 6-wk washout before commencing treatment with the second agent. Studies of renal function were performed at baseline and upon completion of 6 wk of treatment. The placebo was saccharin 1 tablet/d (similar in appearance to amiloride) for the first 2 wk and 2 tablets for the remaining 4 wk; 5 mg/d of amiloride (APS Berk Pharmaceutical, Leeds, United Kingdom) was given for the first 2 wk and increased to 10 mg/d for the final 4 wk of the crossover trial.

Clinical Studies

Following an overnight 12-h fluid restriction, subjects presented to the Clinical Research Area (Department of Medicine, Dunedin School of Medicine or Department of Psychologic Medicine, Christchurch School of Medicine). Height and weight were recorded. Participants were instructed to take their routine medications following baseline blood samples on the day of study. Baseline blood samples for plasma osmolality, sodium, creatinine, lithium, and AVP were taken. Baseline urine samples for creatinine, AQP2, cAMP levels, and osmolality were collected. Participants then received a standard dose of 40 μg dDAVP (desmopressin, a synthetic analogue of AVP-AFT Pharmaceuticals, Clin J Am Soc Nephrol 3: 1324–1331, 2008 Lithium, Amiloride, and Urinary AQP2 Excretion 1325

Table 1. Demographic data of all participants

<table>
<thead>
<tr>
<th></th>
<th>Lithium-treated group</th>
<th>Lithium-naive group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female:male</td>
<td>26:19</td>
<td>36:6</td>
</tr>
<tr>
<td>Age (yr) [mean (range)]</td>
<td>48 (20-68)</td>
<td>33 (16-66)</td>
</tr>
<tr>
<td>Duration of therapy (yr)</td>
<td>22 (11-28)</td>
<td></td>
</tr>
<tr>
<td>Alternative and/or supplementary drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs with serotonergic effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRIs</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Tricyclics</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Drugs with no serotonergic effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAOIs</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Antiepileptic agents</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

SSRIs, selective serotonin reuptake inhibitors (fluoxetine, citalopram); tricyclics (doxepin, imipramine, clomipramine, nortriptyline, dothiepin); MAOIs, monoamine oxidase inhibitors (tranylcypromine, moclobamide); benzodiazepines (diazepam, clonazepam); antiepileptic agents (carbamazepine, valproate); antipsychotics (clozapine, olanzepine, quetiapine, prochlorperazime, starazine).
Auckland, New Zealand) intranasally. Water intake was restricted to 500 ml over the next 6 h. All urine passed was collected and aliquots analyzed for creatinine, osmolality, AQP2, and cAMP levels.

Plasma osmolality, creatinine, lithium, and sodium as well as urinary osmolality and creatinine were measured by standard automated laboratory assays (Healthlab Otago, Dunedin Hospital, Canterbury Health Laboratories, Christchurch Hospital, New Zealand). Urinary cAMP was assayed using a cAMP kit (Biotrak Assay System, Uppsala, Sweden; RPA 509) by the Cardioendocrine Research Group, Department of Medicine, Christchurch School of Medicine, and plasma AVP was measured by an in-house radioimmunoassay (19) (Endolab, Christchurch Hospital, New Zealand).

**Measurement of AQP2 in the Urine**

Urinary AQP2 levels were measured using a chemiluminescent assay (14,15). Briefly, 15 ml of urine was spun (500 × g) for 10 min to remove cell debris, and the supernatant was then concentrated in a Centriplus YM 10 filter (Millipore, Billerica, MA) following the manufacturer’s instructions. Laemmli loading buffer was added to the samples in the ratio of 2:1 and the samples denatured at 95°C for 5 min. Samples equivalent to 4 mol urinary creatinine were applied to membranes (Immobilon PDVF, Millipore) in a BioDot apparatus (Bio-Rad, Hercules, CA). The membranes were blocked for one hour in 5% nonfat dried milk in Tris-buffered saline (200 mmol/L Tris, 73 mmol/L NaCl, pH 7.6, with 1% Tween 20 (vol/vol)). After washing, the membranes were incubated overnight at 4°C with a 1:2500 dilution of rabbit antiserum raised against a synthetic peptide made from the C-terminal end of human AQP2 (amino acids 257 to 271: VELH-SPQALPRGTKA) (Chiron Mimetopes, Melbourne, Australia). After further washing, the membranes were incubated in the secondary antibody at 1:3000 dilution of goat antirabbit IgG coupled to HRP (PO488, Dako Denmark A/S, Glostrup, Denmark). Antigen-antibody reactions were detected.
Lithium, Amiloride, and Urinary AQP2 Excretion  
1327

by enhanced chemiluminescence (Supersignal West Pico, Pierce Chemical, Rockford, IL) according to the protocol supplied by the manufacturer.

To quantitate the amounts of AQP2 in the urine, the synthetic immunogenic peptide (Chiron Mimotopes) was cross-linked to bovine serum albumin using a protein-protein cross linking kit (P6305, Invitrogen, Carlsbad, CA). Membranes were prepared as outlined above, with the cross-linked synthetic peptide used as a standard. The resulting x-ray film was scanned densitometrically (GS-700, Bio-Rad) and analyzed using Molecular Analyst software. A standard curve (serial dilutions of the peptide) using the cross-linked protein versus densitometric readings was made. Urinary AQP2 levels were recorded relative to urinary creatinine (fmol/μmol).

Baseline blood samples were used for DNA extraction using the Hi Pure PCR template preparation kit (Roche, Basel, Switzerland). DNA fragments encoding the whole of the AVPR gene, exon 1, exons 2 to 3, and exon 4 of AQP2 were amplified using the Fast Start Hi Fidelity PCR system (Roche), approximately 125 ng genomic DNA, and sets of primers (20–22). For each set of polymerase chain reaction (PCR) reactions, a sample lacking DNA template was included, and no amplification was observed in these samples. One fifth of each PCR reaction was analyzed by agarose gel electrophoresis to confirm that a single product of the correct size was amplified. The remainder of the PCR product was purified using the Hi Pure PCR purification kit (Roche), and the concentration determined by absorbance at 260 nm. For DNA sequencing reactions, 2 ng 100 bp of PCR product and 3.2 pmol of primer were used, and sequencing was performed by the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand). Sequencing of AVPR PCR products was performed using the 5’ primer (20–24) and a reverse primer: CTG TGC TGG GCC ATC CCT CT. AQP2 exon 1 PCR products were sequenced using: AGAGC GAG TGC CCG GAG, AQP2 exon 2 to 3 sequenced using the 5’ primer for exon 2, (24) and exon 4 was sequenced using the 5’ primer (24).

Statistics
Values are means ± SEM, r values, and statistical values (analysis of variance) were calculated with Kaleidagraph (Synergy Software, Reading, PA).

Results
All 87 subjects (45 on lithium therapy) completed the first study, with no reported side effects. Participants not on lithium therapy (lithium-naïve) had normal urinary concentrating ability, giving an appropriate rise in urinary osmolality following overnight water deprivation, and a further rise following dDAVP administration (Figure 1). Participants on lithium therapy demonstrated a variable ability to concentrate their urine and for subsequent analysis (Figure 1; Table 1) were grouped into tertiles (25) according to their initial overnight urinary osmolality (overnight urinary osmolality >750 mOsm/kg, 750 to 300 mOsm/kg, or <300 mOsm/kg).

Urinary excretion of AQP2 increased following administration of dDAVP in the non–lithium-treated group (Figure 1B). The reduction in maximal urinary concentrating ability in the lithium-treated groups (Figure 1A) was associated with a lesser increase in AQP2 excretion (Figure 1B) and urinary cAMP excretion (Figure 1C). The correlation between urinary AQP2 excretion and urinary cAMP excretion in all individuals was significant (r = 0.85).

The reduction of urinary concentrating ability as well as the decreased excretion of urinary AQP2 and cAMP correlated with the duration of lithium therapy (r = 0.63 for AQP2 and 0.52 for cAMP; Figure 2). For those individuals who had been on lithium for 20 yr or more, overnight osmolality had fallen to 650 ± 81 mOsm/kg, urinary cAMP excretion had fallen to 331 ± 69 pmol/μmol creatinine, and urinary AQP2 excretion had fallen to 80 ± 31 fmol/μmol creatinine (Figure 2). The ability to respond to dDAVP had correspondingly decreased. In those individuals not on lithium, urinary osmolality (958 ± 51 mOsm/kg) and urinary cAMP excretion (286 ± 14 pmol/μmol creatinine) were not affected by the duration of their therapy.

Participants in the second study ranged in age from 37 to 71 yr, and all had at least partial NDI induced by their lithium therapy. The duration of lithium therapy before the study ranged from 8 to 34 yr (Table 3). All 11 subjects completed the study with no reported side effects. Plasma lithium concentrations were not modified by 6 wk of amiloride therapy and remained within the accepted therapeutic range. There was no evidence of any change in the mood status of the participants. The plasma osmolality, creatinine, and sodium concentrations were similar in both arms of the study (Table 4).

After 6 wk of amiloride therapy, there was a significant improvement in urinary osmotic concentration following dDAVP compared with baseline (164.5% ± 8% increase, P ≤ 0.05) with an associated increase in urinary AQP2 excretion (104% ± 32% increase; Figure 3). Urinary cAMP excretion was more variable. There was no change in urinary parameters following placebo therapy (Figure 3).

Recently, there have been a number of reports of missense mutations for aquaporins and vasopressin receptors that are associated with the development of NDI (20–24). We therefore screened all participants in the cross-sectional study to try and identify any possible missense mutations coding for aquaporin or vasopressin receptors. Genomic DNA was purified from the blood samples, the AVP receptor and AQP2 exons were amplified, and the coding regions were analyzed by DNA sequencing as described in Materials and Methods. No mutations in either the AVP receptors or AQP2 genes were found in any of the samples.

Discussion
The first study, a cross-sectional investigation of individuals on psychotropic therapy for management of their mood disorder, clearly demonstrated a correlation between duration of lithium therapy, impaired urinary concentrating ability, and reduced levels of urinary AQP2 excretion. Individuals with a treated mood disorder, who had not been exposed to lithium, demonstrated a normal ability to concentrate their urine following overnight water deprivation and subsequent dDAVP stimulation. The resulting increase in urinary osmolality was accompanied by an appropriate increase in urinary AQP2 excretion. Movig et al. had suggested that the combined use of lithium and serotonergic antidepressants created a threefold higher risk of polyuria compared with lithium alone (8). In this study, the use of serotonergic drugs was equally distributed
Figure 1. Urine osmolality (A), cAMP excretion (B), and AQP2 excretion (C) of participants, grouped into tertiles according to their ability to concentrate urine, after overnight water deprivation and administration of desmopressin (dDAVP), 40 μg intranasally; n = 87. Data mean ± SEM. *P < 0.05, **P < 0.01 compared with the lithium-naive group. P < 0.001, difference between baseline and maximum in each case. n = 2 for >300 mOsm/kg tertile.

Figure 2. Urinary osmolality, cAMP excretion, and AQP2 excretion of participants, grouped according to the length of exposure to lithium, after overnight water deprivation and administration of desmopressin (dDAVP), 40 μg intranasally; n = 87. Data mean ± SEM. *P < 0.05, **P < 0.01 compared with the lithium-naive group. P < 0.001, difference between baseline and maximum in each case.
between lithium-naive individuals and those on lithium therapy. There was no evidence of a possible serotonergic effect on urinary concentrating ability. Similarly, the suggestion that long-term psychiatric disorders, irrespective of the psychotropic medication used, may produce impaired urinary concentrating ability (8) is not supported by the results of this study.

This study, in common with previous reports (2,18), demonstrates that long-term lithium exposure produces a reduction in urinary concentrating ability, which is related in part to a blunting of the response to AVP. The decreased urinary excretion of AQP2 infers a reduction in insertion of AQP2 into the apical membrane of the collecting duct cells, as has been demonstrated experimentally in rats (9,26–28). Previously, we had demonstrated that short-term exposure to lithium in healthy participants produced a small but significant reduction in urinary concentrating ability and reduction in urinary AQP2 (17). Clearly, in those individuals in the present study who have been on long-term lithium therapy, there is a far greater degree of impaired urinary concentrating ability, and this is associated with reduced urinary AQP2 excretion. This was most marked in the two individuals with frank NDI whose urinary AQP2 concentrations were very low.

Urinary AQP2 excretion was linearly correlated with urinary cAMP over the entire study sample, ranging from frank NDI, through partial NDI, to lithium-naive individuals who exhibited normal renal concentrating ability (r = 0.85). Nielsen et al. have demonstrated, in animal models, that lithium inhibits AVP-stimulated insertion of AQP2 into the apical membrane due to inhibition of adenyl cyclase, which is normally activated following binding of AVP to the V2 receptor (11,12,26). On the other hand, Deen et al. have demonstrated that cAMP and AQP2 downregulation may be at least partially independent of each other (28). The changes in urinary cAMP excretion found in the present study were more variable than the changes in urinary AQP2 excretion but still exhibited an apparent trend toward downregulation (Figures 1 and 2) as the ability to respond to dDAVP decreased.

At what point, or in which individuals, lithium-induced changes in urinary concentrating ability become irreversible is not known. Because lithium is widely used for the treatment of mood disorders, this study indicates the need for a better understanding of the renal effects of long-term lithium therapy.

The results of the second study, the first randomized placebo-controlled trial of amiloride, further confirms the results of earlier open-label studies of amiloride, demonstrating that amiloride can alleviate or ameliorate lithium-induced NDI (2,18). We infer that amiloride, by binding to the epithelial sodium channel, ENaC in the renal collecting duct, abolishes the uptake of lithium by this segment. This in turn releases the lithium-induced inhibition of cAMP generation by AVP, more AQP2 being transported to the cell surface, allowing an improvement in renal concentrating ability. This is supported by the measured increase in AQP2 excretion following amiloride. Excretion of cAMP was very variable and did not parallel the changes in AQP2 excretion. In another cross-sectional study, Wilting et al. (29) studied 20 patients on lithium and demonstrated a partial correlation between urinary cAMP and urine osmolality but did not find a correlation between urinary AQP2 and urinary cAMP or urinary osmolality. This probably reflects the different study protocols. Wilting et al. (29) measured dDAVP-stimulated urinary concentrating ability after having

Figure 3. Maximum percentage change in urinary osmolality (A), AQP2 excretion (B), and cAMP excretion (C) of participants maintained on lithium, in a double-blind amiloride/placebo crossover trial; n = 11. Data mean ± SEM. ***p < 0.05, compared with baseline.
given the subjects a water load, which would have suppressed the normal urinary osmotic gradient, whereas our subjects were examined under conditions of maximal urinary concentrating ability, with dDAVP being administered after a 12-h overnight fluid deprivation.

The variable results in urinary cAMP measured in the present studies would tend to support the observation made by Deen et al. (28) that alternative pathways for AQP2 insertion exist. However, this study does not provide any mechanistic explanation for the changes observed. In a recent study, we have demonstrated that amiloride administered to rats with established lithium-induced NDI restores the medullary osmotic gradient, with increased AQP2 and urea transporter (UT-A1) expression (30). The increased expression of UT-A1 was in the inner medulla well away from the principal cells of the collecting duct (the location of ENaC and amiloride action). UT-A1 expression is in part regulated by AVP. This would suggest that amiloride and/or inhibition of lithium uptake may have indirect effects in addition to the specific changes well documented in the collecting tubules (26, 27). This is also supported by the observation that, although urinary AQP2 excretion was increased, it did not parallel the increase in urinary osmolality.

Because not all individuals taking lithium therapy develop NDI, we further postulated that there may be a genetic predisposition to the development of lithium-induced NDI. Recently, there have been a number of reports of missense mutations for aquaporins and vasopressin receptors that are associated with the development of NDI (20–24). We therefore screened all 87 participants to try and identify any possible missense mutations coding for aquaporin or vasopressin receptors. No mutations in either the AVP receptors or AQP2 genes were found in any of the samples. Clearly, the relatively small size of our study population (87 participants) limits the ability to identify any possible gene defect. Nevertheless, this screening would make it highly unlikely that there is a specific defect in those genes studied to explain the susceptibility to lithium-induced impairment of urinary concentrating ability in the majority of individuals.

Conclusion
This study has demonstrated that lithium-induced NDI is associated with reduced urinary AQP2 excretion. The impaired concentrating ability and decreased urinary AQP2 excretion correlate with the duration of exposure to lithium. Other psychotropic agents are not associated with an impaired urinary concentrating ability. Amiloride partially restores the urinary concentrating ability, and this is associated with increased urinary AQP2 excretion. Further studies are required to clearly define the mechanisms of how amiloride modifies lithium-induced NDI.

Acknowledgments
The authors thank members of the Otago Bipolar Group for participating in this work and Dr Bernhard Schmitt for helpful criticism of the manuscript. The research was supported by the National Kidney Foundation of New Zealand and the Health Research Council of New Zealand (Renal effects of lithium therapy in bipolar disorders). S.W. was a summer research student supported by the National Kidney Foundation of New Zealand. Christchurch subjects were participating in a Health Research Council of New Zealand-funded project (Psychotherapy for young people with bipolar disorder).

Disclosures
None.

References

Access to UpToDate on-line is available for additional clinical information at http://www.cjasn.org/