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Lithium-induced reduction in urinary concentrating ability and urinary aquaporin 2 (AQP2) excretion in healthy volunteers

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Lithium-induced reduction in urinary concentrating ability and urinary aquaporin 2 (AQP2) excretion in healthy volunteers.

Background. Lithium therapy is associated with the development of nephrogenic diabetes inspidus. Experimentally, lithium inhibits arginine vasopressin (AVP)-stimulated translocation of cytoplasmic aquaporin 2 (AQP2) to the apical membrane. Clinically, the actions of lithium on renal tubular function are less clearly established. This study examined the effects of four weeks of lithium therapy on desmopressin (dDAVP)-stimulated urinary concentrating ability in healthy volunteers.

Methods. Eleven healthy volunteers underwent baseline urinary concentrating ability studies which were repeated following 4 weeks therapy with lithium carbonate (250 mg twice a day). Urinary osmolality, urinary AQP2 and cyclic adenosine monophosphate (cAMP) levels were measured following overnight fluid deprivation and after the administration of 40 μg of dDAVP. Baseline values were compared with results after 4 weeks of lithium therapy.

Results. Four weeks of lithium therapy reduced dDAVP-stimulated urinary concentrating ability (996 ± 27 to 945 ± 26 mOsm/kg) ($P < 0.05$) and this was associated with significant reduction in urinary AQP2 excretion (99.2 ± 10.0 to 77.8 ± 7.4 fmol/μmol creatinine) ($P < 0.05$) and urinary cAMP excretion (3188 ± 376 to 2212 ± 378 units) ($P < 0.01$).

Conclusion. Four weeks of lithium therapy in healthy volunteers produced a small but significant reduction in dDAVP-stimulated urinary concentrating ability, which appears to be mediated by the inhibition of AVP-stimulated translocation of cytoplasmic AQP2 to the collecting tubule apical membrane via inhibition of adenyl cyclase.

Lithium therapy is a major therapeutic agent used to treat patients with various mood disorders. However, it has been associated with several different forms of renal injury. In an analysis of several studies published from 1979 to 1986, comprising 1172 patients [1], the most prevalent renal effect of lithium was impairment of renal concentrating ability, which was estimated to be present in at least 54% of 1105 unselected patients on chronic lithium therapy. This defect translated into overt polyuria and polydipsia in up to 20% of unselected cases [1]. Initially, the decreased urinary concentrating ability is largely reversible following cessation of lithium. Of greater concern is the development of a progressive impairment of urinary concentrating ability [nephrogenic diabetes inspidus (NDI)] in patients on long-term maintenance lithium therapy, which is resistant to the actions of arginine vasopressin (AVP) (antidiuretic hormone). This functional lesion is not usually reversible and is associated with a chronic focal interstitial fibrosis in renal biopsy [2, 3]. The lithium-induced interstitial fibrosis may be progressive leading to end-stage renal failure [3].

The ability of the mammalian kidney to produce a concentrated urine is the function of two processes. First, there is the creation of a hyperosmotic medullary interstitium, and second there is the insertion of aquaporins into the apical [aquaporin 2 (AQP2)] and basolateral membranes (AQP3 and AQP4) of the collecting tubules to allow the reabsorption of water down a concentration gradient. Of these, AQP2, confined to the apical regions of the principal cells of the collecting tubules, is the primary target for short-term regulation of collecting duct permeability by the antidiuretic hormone (AVP) [4, 5]. Failure of such regulation, which may occur as a result of a number of pathophysiologic conditions, leads to disorders of water balance, manifest clinically by an inability to concentrate the urine with associated nocturia and polyuria [4, 5]. If not controlled, this has the ability to further compromise renal function.

Lithium is likely to enter collecting duct cells via the luminally located epithelial sodium channel (ENaC) since this channel has a higher permeability for lithium compared to sodium (reviewed in [6]). Once inside collecting duct cells, lithium influences both sodium and water handling pathways. Chronic lithium treatment in

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rats reduces amiloride-sensitive (ENaC) sodium current and increases urinary sodium excretion [7]. This appears to be due to down-regulation of ENaC subunit proteins within the cells of the cortical and outer medullary collecting duct [8]. 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 AQP2 to the apical membrane. This may be due to lithium competing with magnesium, inhibiting magnesium-dependent proteins that activate AVP-sensitive adenyl cyclase [9]. AVP binds to its V_2 receptor stimulating adenyl cyclase and cyclic adenosine monophosphate (cAMP) formation, which activates protein kinase A. Protein kinase A phosphorylates cytoplasmic AQP2, which is essential for its translocation and insertion into the apical membrane. Insertion of AQP2 into the apical membrane then allows water permeability and reabsorption in the cortical collecting tubule. Failure of AQP2 insertion leads to an excess of water delivered to the medullary tubule, which exceeds the tubules capability to reabsorb water [4, 5]. The net effect is polyuria.

Although there have been a number of experimental studies investigating the actions of lithium, we report the first study investigating the acute short-term (4 weeks) effects of lithium therapy on urinary AQP excretion and water concentrating ability in healthy volunteers. The aim of this study was to measure the changes in AVP-stimulated urinary concentrating ability and urinary AQP excretion following 4 weeks of lithium carbonate therapy. Baumgarten et al [10] have shown measurement of urinary AQP2 excretion to be a useful approach to study urinary concentrating defects and we have confirmed the validity of urinary AQP measurement in response to water deprivation and desmopressin (dDAVP) administration in normal volunteers [11].

METHODS

Eleven healthy volunteers (seven males and four females), with no known medical problems and taking no medication, whose ages ranged from 20 to 63 years took part in the study, after giving written informed consent. The study was approved by the Otago Ethics Committee. Participants' demographic data are presented in Table 1. Following baseline studies of urinary concentrating ability, each participant took lithium carbonate 250 mg twice a day for 28 days. The urinary concentrating ability study was then repeated.

Clinical studies

Following an overnight 12-hour fluid restriction, subjects presented to the Clinical Research Area (Department of Medicine). Height and weight was recorded. Baseline blood samples for plasma osmolality, sodium, creatinine, lithium, and AVP were taken. Baseline urine was collected and analyzed for creatinine, AQP2, cAMP levels, and osmolality. Subjects then received a standard dose of 40 μg dDAVP (a synthetic analogue of AVP) intranasally. Water intake was restricted to 500 mL over the next 6 hours. All urine passed was collected and aliquots analyzed for urinary creatinine, osmolality, AQP2, and cAMP levels.

Table 1. Demographics of participants

Age	Mean 36.5 years (range 20–63 years)
Male:female	7:4
Plasma creatinine	Mean 85 μmol/L (range 67–104 μmol/L)
Lithium concentrations at completion of 4 weeks	Mean 0.45 mmol/L (range 0.3–0.8 mmol/L)

Plasma osmolality, creatinine, lithium, and sodium as well as urinary osmolality and creatinine were measured by standard automated laboratory assays (Healthlab Otago, Dunedin Hospital, New Zealand).

Urinary cAMP was assayed using a cAMP kit supplied by BIOTRAK Assay System (code RPA 509; Amersham Life Sciences, Sydney, Australia) and plasma AVP was measured by an in-house radioimmunoassay [12] (Endolab) (Christchurch Hospital, Christchurch, New Zealand).

Urinary AQP2 levels were measured using a validated chemiluminescent assay [11]. Briefly, 15 mL of urine was spun ($500 \times g$) for 10 minutes to remove cell debris and the supernatant was then concentrated in a Centriplus YM 10 (Amicon, Millipore, Bedford, MA, USA) filter 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 minutes. Samples equivalent to 4 μmol urine creatinine were applied to membranes [Immobilon polyvinylidine difluoride (PDVF) (Millipore, Bedford, MA, USA) in a BioDot apparatus (Bio-Rad, Hercules, CA, USA). The membranes were blocked for 1 hour in 5% nonfat dried milk in Tris-buffered saline (TBS) [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 1:2500 dilution of rabbit antiserum raised against a synthetic peptide made from the C- terminal end of human AQP2 (amino acids 257-271 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 horseradish peroxidase (HRP) (DAKO, PO488, Glostrup, Denmark). Antigen-antibody reactions were detected by enhanced chemiluminescence (Supersignal West Pico; Pierce, Rockford, IL, USA) according to the protocol supplied by the manufacturer.

In order to quantitate the amounts of AQP2 in the urine, synthetic peptide (Chiron Mimetopes, Melbourne) was cross-linked to bovine serum albumin using a protein-protein cross linking kit (P6305) (Molecular Probes,