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Urine osmolality, cyclic AMP and aquaporin-2 in urine of patients under lithium treatment in response to water loading followed by vasopressin administration

Ingeborg Wilting [a,b], Ruben Baumgarten [c], Kris L.L. Movig [d], Jan van Laarhoven [e], Alfred J. Apperloo [f], Willem A. Nolen [g], Eibert R. Heerdink [a], Nine V.A.M. Knoers [h], Antoine C.G. Egberts [a,i,*]

[a] *Utrecht University, Faculty of Science, Utrecht Institute for Pharmaceutical Sciences, Division of Pharmacoepidemiology and Pharmacotherapy, Utrecht, The Netherlands*
[b] *Department of Clinical Pharmacy, TweeSteden hospital and St Elisabeth Hospital, Tilburg, The Netherlands*
[c] *Department of Clinical Chemistry, Hospital Gelderse Vallei, Ede, The Netherlands*
[d] *Department of Clinical Pharmacy, Medisch Spectrum Twente, Enschede, The Netherlands*
[e] *Department of Psychiatry, St Elisabeth Hospital, Tilburg, The Netherlands*
[f] *Department of Nephrology, St Elisabeth Hospital, Tilburg, The Netherlands*
[g] *Department of Psychiatry, University Medical Center Groningen, University of Groningen, The Netherlands*
[h] *Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*
[i] *Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht, The Netherlands*

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Abstract

Lithium is the drug that is most frequently associated with acquired nephrogenic diabetes insipidus (NDI). The exact mechanism of lithium-induced NDI in man is unknown. The aim of the present study was to investigate the kidney response to minimal and maximal stimulation of the kidney urine concentrating mechanism by measuring urine osmolality, and urine levels of cAMP and AQP-2 in urine of patients under long-term lithium treatment.

Twenty patients under long-term lithium treatment were included. The kidney urinary 3′,5′-cyclic adenosine monophosphate (cyclic AMP), aquaporin-2 levels and urine osmolality were determined during a situation of minimal kidney urine concentrating activity (induced by water loading) and during a situation following maximal stimulation of kidney urine concentrating activity (induced by 1-desamino-8-D-arginine-vasopressin).

Patients were classified as NDI, partial NDI and non-NDI based on maximal reached urine osmolality. The partial correlation (r) between urinary cyclic AMP levels (mol/l) and urine osmolality was 0.94 ($P<0.001$). No significant correlation was observed between urinary aquaporin-2 levels (mol/mol creatinine) and osmolality nor between urinary cyclic AMP and aquaporin-2 levels. The rise in urinary cyclic AMP but not aquaporin-2 levels upon 1-desamino-8-D-arginine-vasopressin administration after water loading significantly differed between the three categories, decreasing with increasing NDI category.

In conclusion we found that in lithium-induced kidney urine concentrating deficit in man, the cyclic AMP generation in response to 1-desamino-8-D-arginine-vasopressin administration after water loading, is impaired. It remains to be elucidated whether principal cells, G-proteins or adenylate cyclase e.g. are the major targets for the mechanism underlying lithium-induced NDI in man.

Keywords: Lithium; Cyclic AMP; Aquaporin-2; Nephrogenic diabetes insipidus; Vasopressin

1. Introduction

Long-term use of lithium salts, one of the first choice agents for the treatment of bipolar disorders, is often complicated by

* Corresponding author. Utrecht Institute for Pharmaceutical Sciences Division of Pharmacoepidemiology and Pharmacotherapy PO Box 80 082, 3508 TB Utrecht, The Netherlands. Tel.: +31 30 2537324; fax: +31 30 2539166.
E-mail address: a.c.g.egberts@uu.nl (A.C.G. Egberts).

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side effects such as tremor, hypothyroidism and nephrogenic diabetes insipidus (NDI), often resulting in polyuria (24-hour urine volume ≥3 l). A diminished kidney urine concentrating ability in response to adequate release of the antidiuretic hormone arginine vasopressin (AVP) occurs in approximately 54% (Boton et al., 1987) of the long-term lithium users, whereas in about 12% forthright NDI (maximal urine osmolality <350 mOsm/kg) develops (Bendz and Aurell, 1999; Mukhopadhyay et al., 2001). Although the exact mechanism of lithium-induced NDI is at present unknown, it has been demonstrated that the risk for lithium-induced NDI is associated with duration of treatment, serum level and cumulative dose (Gitlin, 1999).

In normal physiology AVP, that is released from the posterior pituitary gland in response to an increased serum osmolality or a decreased effective circulating volume, stimulates the basolaterally located kidney vasopressin 2 receptor. Vasopressin 2 receptor stimulation subsequently results in G-protein induced activation of adenylate cyclase, stimulating 3′,5′-cyclic adenosine monophosphate (cyclic AMP) formation. Next, cyclic AMP stimulates protein kinase A, followed by activation of its catalytic subunit, in turn resulting in phosphorylation of aquaporin-2 in the cytoplasmic vesicles. Finally, the cytoplasmic vesicles containing aquaporin-2 protein fuse with the apical membrane of the ductal tubular cells, rendering this normally water tight membrane permeable for water. As a consequence of the osmotic force of the hypertonic interstitium, water is then reabsorbed from pro-urine. Finally, water flows into the main circulation through basolaterally located aquaporin-3 and aquaporin-4 channel proteins (Deen et al., 2000; Nielsen et al., 2002; Oksche and Rosenthal, 1998). Studies have demonstrated that both aquaporin-2 and cyclic AMP are detectable in urine of healthy volunteers (Baumgarten et al., 2000; Elliot et al., 1996; Ishikawa et al., 1998; Kanno et al., 1995; Knoers and van Os, 1995; Pasel et al., 2000; Saito et al., 1997). Urinary excretion of aquaporin-2 has been suggested to be a surrogate marker of AVP stimulated insertion of aquaporin-2 in the ductal tubular apical membrane (Al-Dameh et al., 2003; Elliot et al., 1996; Ishikawa et al., 1998; Kanno et al., 1995). In healthy volunteers a correlation was established between aquaporin-2 and both an increased AVP plasma level and exogenous vasopressin 2 receptor stimulation (Al-Dameh et al., 2003; Baumgarten et al., 2000). Small studies with patients with either central nephrogenic diabetes insipidus or patients with congenital or lithium-induced NDI, demonstrated either minimal or no 1-desamino-8-D-arginine-vasopressin-induced aquaporin-2 excretion in urine (Ishikawa et al., 1998; Kanno et al., 1995; Saito et al., 1997).

Presently, it is unknown which mechanism is exactly responsible for the occurrence of NDI in patients under long-term lithium treatment and what is exactly the target for lithium. Some studies on the mechanism of lithium-induced NDI in animal models have shown that lithium can impair cyclic AMP generation, possibly by modulating the interaction between G-proteins and adenylate cyclase (Dousa, 1974; Jackson et al., 1980; Nielsen et al., 2002; Yamaki et al., 1991). Other studies have implied the involvement of other mechanisms such as down-regulation of the vasopressin 2 receptor (Hensen et al., 1996), a direct effect on aquaporin-2 (Marples et al., 1995) or on the principal cells (Christensen et al., 2004).

In order to investigate which part of the vasopressin 2 receptor–cyclic AMP–aquaporin-2 cascade is involved in the mechanism of lithium-induced NDI in man, we investigated the kidney response to minimal and maximal stimulation of the kidney urine concentrating mechanism by measuring urine osmolality, and urine levels of cAMP and AQP-2 in urine of patients under long-term lithium treatment.

2. Methods and materials

2.1. Setting

The study was conducted at the psychiatric department of the St. Elisabeth Hospital, in Tilburg; a large teaching hospital located in the South of The Netherlands. The study was performed in accordance with the current revision of the Declaration of Helsinki International Conference on Harmonization guidelines and Good Clinical Practice guidelines (Anonymous, 1982).The medical ethics committee of the St. Elisabeth Hospital approved the study protocol. Each patient gave written informed consent after full explanation of the study, both verbally and in writing.

2.2. Study population

Participants of at least 18 years of age who were under long-term lithium treatment (defined as having been maintained on lithium for at least 2 years prior to inclusion) were selected from a population of 75 patients, in whom the presence of polyuria (24-hour urine volume ≥3 l) had been determined in a previously reported study (Movig et al., 2003). In order to obtain representatives in all of the three respective categories (NDI, partial NDI and non-NDI) we included ten patients with polyuria and ten patients without polyuria.

Patients were not included in the present study if they had kidney function impairment (Glomerular Filtration Rate (GFR) <80 ml/min or proteinuria >0.3 g/l), diabetes mellitus, cardiac disease, hypertension, known pregnancy, or if they were using carbamazepine, oxcarbazepine, (non-steroidal anti-inflammatory drugs) NSAIDs or acetaminophen (chronically more than 3 g/24 h). Carbamazepine and oxcarbazepine can cause hyponatreamia (Sachdeo et al., 2002); non-steroidal anti-inflammatory drugs (NSAIDs) frequently interfere with renal function (Murray and Brater, 1990) as does chronic use of high dose acetaminophen (Plotz and Kimberly, 1981).

2.3. Experiment

Patients were instructed not to drink coffee or tea and not to smoke starting from six Post Meridium (PM) prior to the study day. Caffeine has natriuretic properties in man (Shirley et al., 2002). Nicotine induces endogenous AVP secretion (Chiodera et al., 1997). On the study day patients were first subjected to water loading by drinking 150 ml of water every 15 min for

2.5 h in order to completely block endogenous AVP-mediated renal vasopressin 2 receptor stimulation. Half an hour after this period of water loading patients were administered 40 μg of 1-desamino-8-D-arginine-vasopressin intranasally; in order to induce maximal stimulation of the renal vasopressin 2 receptor. Following 1-desamino-8-D-arginine-vasopressin administration, patients were asked to restrict their fluid intake to a minimum. Throughout the study day patients had to urinate seven times according to the study day schedule (Fig. 1). No blood sampling was performed on the study day. Absence of protein in urine was confirmed for all patients.

2.4. Biochemical analysis

Each urine sample was collected separately and after urine-volume determination, samples were, after a two step concentrating procedure, immediately stored at −80 °C until further analysis. Protease inhibitors were added to the concentrated urine samples, before storage, as previously described (Baumgarten et al., 2000). Urine osmolality, creatinine, urinary cyclic AMP (principally expressed in mol/l) and aquaporin-2 (expressed in mol/mol creatinine) levels were determined in each sample. Urine osmolality was determined by measuring freezing point depression (Gennari, 1984). Creatinine was determined by standard automated laboratory techniques. In accordance with current literature urinary cyclic AMP levels were expressed per volume urine and urinary aquaporin-2 levels were expressed per mol creatinine (Bedford et al., 2003; Walker et al., 2005).

Quantification of cyclic AMP was performed in a competitive protein-binding assay using a Radio Immuno Assay (RIA) for quantification and was performed according to the instructions provided by the manufacturer (DPC).

Aquaporin-2 was measured according to a previously described method involving semi-quantitative immunoblot analysis, after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The only adaptation from the original method was substitution of the primary antibody into a commercially available rabbit anti-aquaporin-2 antibody (BDPharmingen) (Baumgarten et al., 2000).

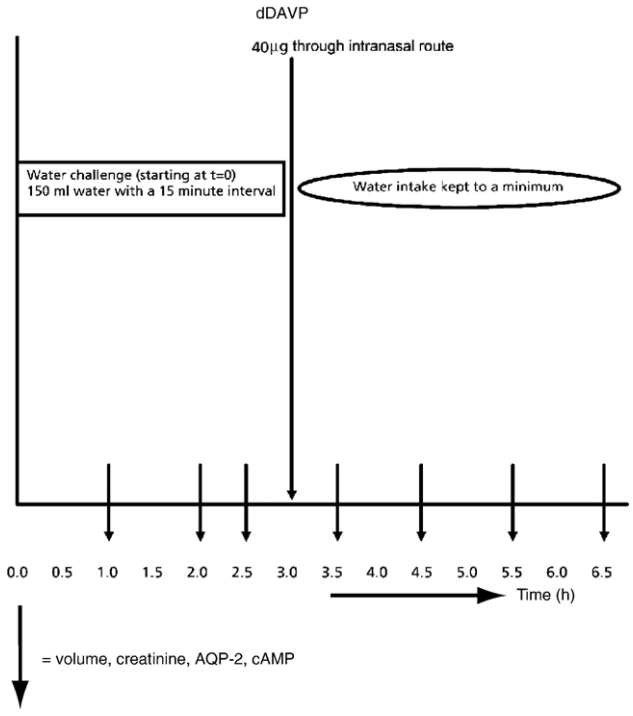

Fig. 1. Schematic representation of study day. Upon presentation at the lithium clinic patients were asked to urinate. Subsequently patients were asked to drink 150 ml of water every 15 min for 2.5 h. Half an hour after the water loading was terminated, patients were administered 40 μg of 1-desamino-8-D-arginine-vasopressin through intranasal route (long arrow). Starting from 3 h prior to 1-desamino-8-D-arginine-vasopressin administration until 3.5 h after 1-desamino-8-D-arginine-vasopressin administration patients were asked to urinate at specific times (small arrows). For each urine sample separately volume, osmolality, creatinine, cyclic AMP and aquaporin-2 levels were determined.

2.5. Additional clinical data

For each patient a pharmacy drug dispensing record, starting at least 1 year prior to the study day, was obtained. From this record concomitant drug use and lithium dose were determined. For each patient the cumulative lithium dose, the most recent lithium serum level prior to the study day and duration of lithium use were obtained from the medical record.

2.6. Data analysis

For each patient we obtained seven different urine samples at seven different time-points during the experiment. Subsequently we determined in each of these samples separately urine osmolality, creatinine, urinary cyclic AMP (principally expressed in mol/l) and aquaporin-2 (expressed in mol/mol creatinine) levels. All seven different observations for urine osmolality, urinary cyclic AMP and aquaporin-2 levels were plotted in time for each patient separately. For each individual patient the correlation between urinary cyclic AMP levels and urine osmolality, respectively between urinary aquaporin-2 levels and urine osmolality, correspondingly between urinary cyclic AMP levels and urinary aquaporin-2 levels was determined using the results from the analysis of the seven different urine samples obtained in time during the experiment. Furthermore the partial correlation r for the pooled study population data, taking into account the within subject dependency of the seven separate observations per patient was determined.

In order to evaluate the kidney's urine concentrating response, the maximal rise in urinary cyclic AMP and aquaporin-2 levels was determined for each individual by comparing the situation of maximal vasopressin 2 receptor stimulation (induced by nasal administration of 40 μg 1-desamino-8-D-arginine-vasopressin) to the situation under minimal AVP-mediated endogenous vasopressin 2 receptor stimulation (induced by water loading). Furthermore the kidney's maximal urine concentrating capacity was evaluated using the maximal reached urine osmolality under exogenous vasopressin 2 receptor stimulation. A linear regression analysis

was performed for both the maximal rise in urinary cyclic AMP and aquaporin-2 levels and the individual maximal reached urine osmolality.

Patients were, based on the maximal reached urine osmolality, categorized to one of the following categories; NDI, partial NDI or non-NDI (Mukhopadhyay et al., 2001).

Patients exhibiting a maximal urine osmolality <350 mOsm/kg upon 1-desamino-8-D-arginine-vasopressin administration were assigned to the NDI class. Patients showing a maximal urine osmolality between 350 and 750 mOsm/kg in response to 1-desamino-8-D-arginine-vasopressin administration were assigned to the partial NDI category. Patients displaying a maximal urine osmolality ≥750 mOsm/kg upon 1-desamino-8-D-arginine-vasopressin administration were assigned to the non-NDI group. Whether the maximal rise in urinary cyclic AMP and urinary aquaporin-2 levels differed significantly between the three groups; NDI, partial NDI and non-NDI, was established by performing the non-parametric Kruskal–Wallis test.

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 12.0.

3. Results

Based on maximal urine osmolality after administration of 1-desamino-8-D-arginine-vasopressin, five patients were assigned to the NDI group, ten patients to the partial NDI group and five patients to the non-NDI group. A typical example of a patient suffering from lithium-induced NDI and a typical example of a patient not suffering from lithium-induced NDI are shown in Fig. 2A and B.

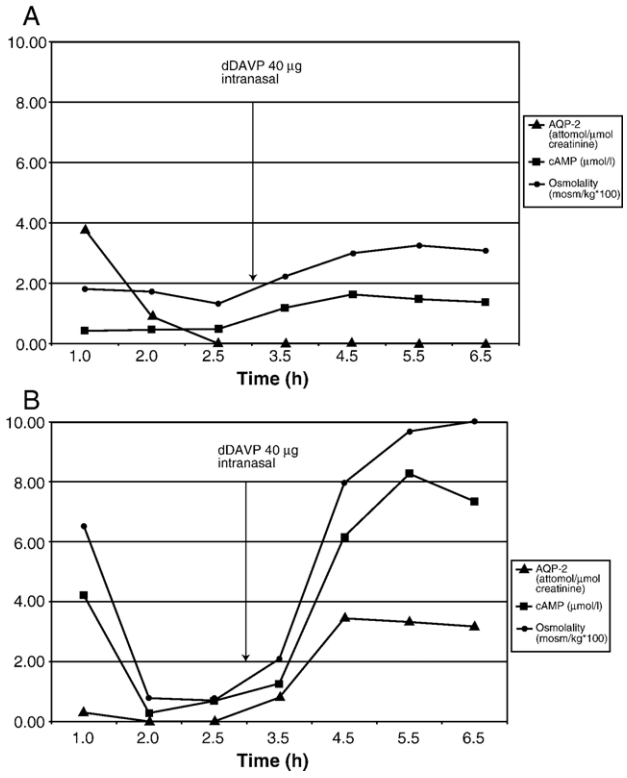

Fig. 2. A: Example of a typical NDI patient; B: example of a typical non-NDI patient.

Table 1
Patient characteristics

	NDI (n=5)	Partial NDI (n=10)	Non-NDI (n=5)
Age (years) mean (range)	58 (50–62)	52 (40–65)	49 (35–68)
Female gender (%)	4 (80%)	8 (80%)	3 (60%)
Polyuria n (%)	4 (80%)	5 (50%)	1 (20%)
Use* (years) mean (range)	17 (12–32)	3.5 (2.5–6.0)	3.9 (2.3–5.4)
Dose (mmol/day) mean (range)	23 (16–42)	25 (16–38)	22 (11–32)
Serum level (mmol/l) mean (range) (most recent prior to the study day)	0.90 (0.7–1.0)	0.74 (0.5–1.0)	0.68 (0.5–1.0)
Total dose[a] (mmol) mean (range)	154,101 (255,949)	31,231 (43,648)	36,544 (46,948)
Smoking (n)	0 (0%)	5 (50%)	2 (40%)

[a] Duration of use (years) (P<0.05) and cumulative dose (mmol) (P<0.05) were shown to differ statistically significant between the three categories (Kruskal–Wallis).

Patient characteristics are reported in Table 1. Duration of lithium use as well as cumulative lithium dose significantly differed between the three categories (Kruskal–Wallis P<0.05). Both duration of lithium use and cumulative lithium dose were significantly higher in those classified as NDI (Table 1), consistent with the reported association between exposure to lithium and the occurrence of kidney urine concentrating deficits in literature (Bendz, 1983; Boton et al., 1987; Gitlin, 1999; Morgan et al., 1982; Mukhopadhyay et al., 2001).

For each individual patient urinary cyclic AMP levels and osmolality measured during the study day were significantly correlated (P<0.05). In 18 of the 20 enrolled patients the correlation r^2 exceeded 0.8 (Fig. 3A). The partial correlation r between urinary cyclic AMP levels and osmolality was 0.94 (P<0.001). The partial correlation for the cyclic AMP corrected for creatinine was 0.229 (P=0.007).

For urinary aquaporin-2 levels no significant correlation with urine osmolality (Fig. 3B) nor urinary cyclic AMP levels could be established neither for the individual data nor for the pooled data (partial correlation r for urinary aquaporin-2 levels and urine osmolality 0.028 (P=0.750)).

The partial correlation r between urinary aquaporin-2 levels and urinary cyclic AMP levels was −0.021 (P=0.813).

In order to investigate whether maximal kidney urine concentrating capacity was correlated to maximal rise in kidney cyclic AMP and/or aquaporin-2 production, we investigated the correlation between the individual maximal reached urine osmolality and the individual maximal rise in urinary cyclic AMP and aquaporin-2 levels. Overall, individual maximal reached urine osmolality was statistically significantly (P<0.001) correlated to the individual maximal rise in urinary cyclic AMP levels (r^2=0.783) (Fig. 4A). When expressing rise

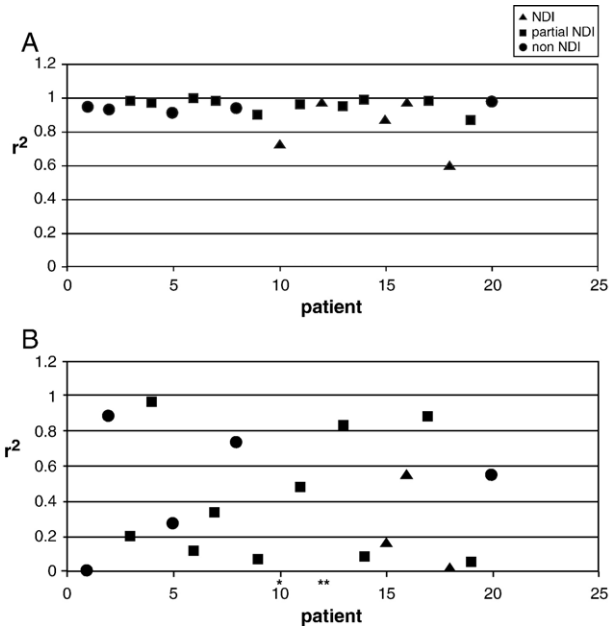

Fig. 3. A: For each patient the correlation between urinary cyclic AMP levels and urine osmolality was determined. Correlation (r^2) between urinary cyclic AMP levels and urine osmolality, for the subsequent urine samples on the study day, in each individual patient is shown. B: For each patient the correlation between urinary aquaporin-2 levels and urine osmolality was determined. Correlation (r^2) between urinary aquaporin-2 levels and urine osmolality, for the subsequent urine samples taken on the study day, in each individual patient is shown. *For patient 12 it was not possible to determine the correlation because of lack of aquaporin-2. **For patient 10 it was not possible to determine the correlation since no aquaporin-2 could be determined because of analytical disturbance in the urine samples.

in cyclic AMP mol/h or corrected for urinary creatinine excretion correlations of $r^2=0.769$ ($P<0.001$) and $r^2=0.002$ ($P=0.858$) were obtained.

The rise in individual urinary aquaporin-2 levels showed a high interindividual variability (Table 2). No significant ($P=0.187$) overall correlation ($r^2=0.100$) could be established for the individual rise in urinary aquaporin-2 levels and the individual maximal reached urine osmolality (Fig. 4B).

In addition we investigated whether there was a statistically significant difference in 1-desamino-8-D-arginine-vasopressin-induced rise in urinary cyclic AMP and aquaporin-2 levels between the three respective NDI categories. We performed a Kruskal–Wallis test of which the results indicated that the 1-desamino-8-D-arginine-vasopressin-induced rise in urinary cyclic AMP levels for the three categories was statistically significantly different ($P=0.006$). Expressing cyclic AMP as mol/h instead of mol/l did not change the results ($P=0.007$).

4. Discussion

A statistically significant correlation was observed between urinary cyclic AMP levels but not urinary aquaporin-2 levels and urine osmolality in response to water loading followed by stimulation with 1-desamino-8-D-arginine-vasopressin in patients under long-term lithium treatment. The severity of the kidney urine concentrating deficit was clearly linked to the degree of diminished 1-desamino-8-D-arginine-vasopressin-induced cyclic AMP generation.

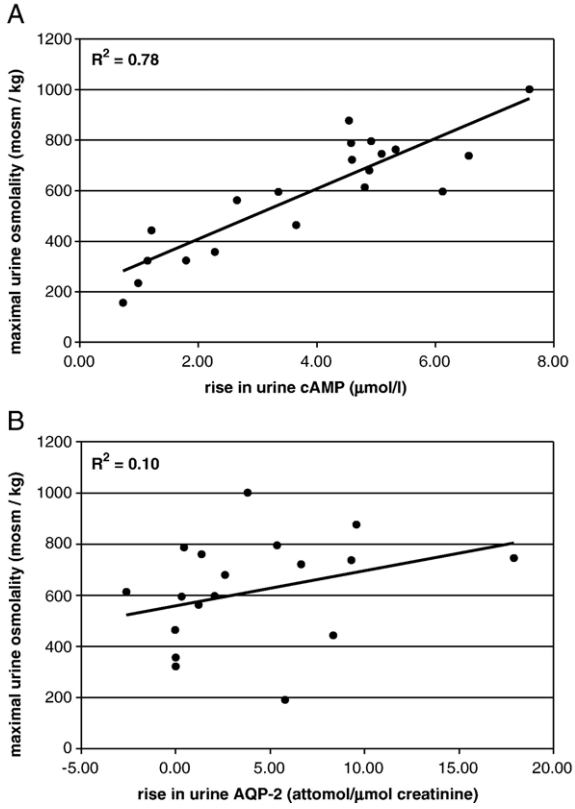

Fig. 4. A: Overall correlation (r^2) between rise in urinary cyclic AMP and maximal reached urine osmolality ($n=20$). For each patient the maximal rise in urinary cyclic AMP levels was determined by taking the difference between the minimal urinary cyclic AMP levels in situation of minimal kidney urine concentrating activity (induced by water loading) and the maximal urinary cyclic AMP levels under a situation following maximal stimulation of kidney urine concentrating activity (induced by 1-desamino-8-D-arginine-vasopressin). The overall correlation between maximal rise in urinary cyclic AMP levels and maximal reached urine osmolality was determined. B: Overall correlation (r^2) between rise in urinary aquaporin-2 levels and maximal reached urine osmolality ($n=19$). For each patient the maximal rise in urinary aquaporin-2 was determined by taking the difference between the minimal urinary aquaporin-2 levels in a situation of minimal kidney urine concentrating activity (induced by water loading) and the maximal urinary aquaporin-2 levels in a situation following maximal stimulation of kidney urine concentrating activity (induced by 1-desamino-8-D-arginine-vasopressin). The overall correlation between maximal rise in urine aquaporin-2 levels and maximal reached urine osmolality was determined. For patient 10 no aquaporin-2 was determined because of analytical disturbances in the urine samples. Upon presentation at the lithium clinic patients were asked to urinate. Subsequently patients were asked to drink 150 ml of water every 15 min for 2.5 h. Half an hour after termination of the water loading patients were administered 40 µg of 1-desamino-8-D-arginine-vasopressin through intranasal route. Starting from 3 h prior to 1-desamino-8-D-arginine-vasopressin administration until 3.5 h after 1-desamino-8-D-arginine-vasopressin administration patients were asked to urinate at specific times (small arrows). For each urine sample separately volume, osmolality, creatinine, cyclic AMP and aquaporin-2 levels were determined. The values are shown at the time of urine sampling in A for a typical NDI patient displaying both inadequate response of urinary cyclic AMP and aquaporin-2 levels in response to maximal stimulation of kidney urine concentrating activity and in B for a typical non-NDI patient displaying both adequate response of urinary cyclic AMP and aquaporin-2 levels in response to maximal stimulation of kidney urine concentrating activity.

Table 2
Kidney urinary concentrating parameters

	NDI ($n=5$)	Partial NDI ($n=10$)	Non-NDI ($n=5$)
Maximal urine osmolality (mOsm/kg) mean (S.D.)	287 (70)	617 (108)	846 (97)
Rise in urinary cyclic AMP[a] level (μmol/l) mean (S.D.)	1.39 (0.64)	4.29 (1.61)	5.39 (1.27)
Mean maximal urine cyclic AMP level (μmol/l) mean (S.D.)	1.88 (0.62)	4.55 (1.54)	5.70 (1.49)
Rise in urinary aquaporin-2[a]> level (attomol/μmol creatinine) mean (S.D.)	1.45 (2.89)[b]	4.58 (6.06)	4.10 (3.63)
Mean maximal urine aquaporin-2 level (attomol/μmol creatinine)	1.45 (2.89)[b]	5.77 (6.32)	4.72 (3.88)

For each urine sample separately volume, osmolality, creatinine, cyclic AMP and aquaporin-2 levels were determined. For each patient maximal reached urine osmolality upon 1-desamino-8-D-arginine-vasopressin administration was determined. For each patient the maximal rise in urinary cyclic AMP and aquaporin-2 levels was determined by taking the difference between the minimal urinary cyclic AMP and aquaporin-2 levels in a situation of minimal kidney urine concentrating activity (induced by water loading) and the maximal urinary cyclic AMP and aquaporin-2 levels in a situation following maximal stimulation of kidney urine concentrating activity (induced by 1-desamino-8-D-arginine-vasopressin). For each of the three categories NDI, partial NDI and non-NDI the mean and standard deviation of the rise in and the maximum urinary cyclic AMP and aquaporin-2 levels were determined as well as the mean and standard deviation of the maximal reached urine osmolality.

[a] Rise in urinary cyclic AMP level ($P<0.001$) but not urinary aquaporin-2 level ($P=0.675$) was shown to differ statistically significant between the three categories (Kruskal–Wallis).

[b] For one individual in the NDI category no aquaporin-2 could be determined due to analytical disturbances.

To our knowledge our study is the first in which, in man, long-term lithium-induced kidney urine concentrating deficits, are linked to an impaired 1-desamino-8-D-arginine-vasopressin-induced cyclic AMP generation.

In a previous study by Walker et al., it was established that 4 weeks of lithium treatment in healthy volunteers resulted in a small but significant reduction in 1-desamino-8-D-arginine-vasopressin-induced kidney urine concentrating capacity, in concurrence with both reduced urinary cyclic AMP and aquaporin-2 levels (Walker et al., 2005). In this study both cyclic AMP and aquaporin-2 were expressed in the same manner as in our study with cyclic AMP as mol/l and aquaporin-2 as mol/mol creatinine. In our study, we investigated the long-term effects of lithium treatment on kidney urine concentrating capacity and urinary cyclic AMP and aquaporin-2 levels, in patients under long-term lithium treatment in daily clinical practice. The observed rise in urinary cyclic AMP levels in our study was statistically significantly less in those exhibiting kidney urine concentrating deficits. Analyzing our results expressing cyclic AMP and AQP-2 respectively as mol/l, mol/mol creatinine or mol/h resulted in a similar trend, revealing significant correlations for urinary excretion of cyclic AMP but not for AQP-2 however the correlation between urinary excretion of cyclic AMP and urine osmolality was less pronounced when expressing cyclic AMP as mol/mol creatinine (results not shown).

It has previously been suggested that lithium acts by inhibiting adenylate cyclase activity in the collecting duct principal cells thereby preventing cyclic AMP formation (Boton et al., 1987; Dousa, 1974; Jackson et al., 1980; Nielsen et al., 2002; Yamaki et al., 1991), which was shown to involve activation of G-inhibitory (Gi) subunits in rats chronically treated with lithium (Yamaki et al., 1991). Furthermore, it has more recently been found, that lithium exhibits a direct toxic effect on the principal cells (Christensen et al., 2004) in animal studies, resulting in a direct loss of vasopressin 2 receptor (Hensen et al., 1996). This could be in accordance with previous reports (Bendz, 1983; Boton et al., 1987; Gitlin, 1999; Morgan et al., 1982; Mukhopadhyay et al., 2001), that show that both duration and total cumulative dose are correlated to the degree of lithium-induced urinary concentrating defect. A significantly higher exposure to lithium (duration of use as well as cumulative dose) was in accordance with previous results established in our NDI group compared to the partial NDI and the non-NDI group in accordance with previously reported studies (Bendz, 1983; Boton et al., 1987; Gitlin, 1999; Morgan et al., 1982; Mukhopadhyay et al., 2001). Both a direct toxic effect of lithium on the principal cells and direct toxic effects on the G-protein or adenylate cyclase could explain these phenomena.

Various other effects of lithium treatment have been found: reduced levels of aquaporin-2-mRNA, next to low Na–K-ATPase mRNA levels (Laursen et al., 2006), and also altered expression of specific acid–base transporters in response to long-term lithium treatment (Kim et al., 2003). The kidney, in lithium-induced NDI, has been shown to be resistant to AVP stimulation of the vasopressin 2 receptor (Kanno et al., 1995).

Based on the limited knowledge of its central therapeutic effects (involving among others effects on the level of the second-messenger cyclic AMP) (Mork et al., 1992) and the above reported renal effects in lithium-treated rats, we hypothesized that lithium is more likely to cause direct interference with the vasopressin 2 receptor–cyclic AMP part of the cascade, rather than to have a direct effect on the aquaporin-2 gene or protein in man. Our results, accordingly, show a diminished urinary cyclic AMP excretion in relation to diminished kidney urine concentrating capacity in patients under long-term lithium treatment. Our findings, which are in accordance with the results described for studies in animals (Boton et al., 1987; Christensen et al., 1985; Dousa, 1974; Jackson et al., 1980; Kwon et al., 2000; Nielsen et al., 2002, Yamaki et al., 1991), indicate that an impaired cyclic AMP generation is, at least in part, responsible for lithium-induced NDI in man. In man low urinary aquaporin-2 levels upon 1-desamino-8-D-arginine-vasopressin administration in lithium-induced NDI have been described (Baumgarten et al., 2000), however without concurrent information on urinary cyclic AMP levels.

The overall results of our study do not show a statistically significant diminished aquaporin-2 generation in those with lithium-induced kidney concentrating deficits. The results of our study therefore do not indicate aquaporin-2 as the primary target in the mechanism underlying lithium-induced NDI. On the other hand, in light of the good correlation established between urine cyclic AMP levels and urine osmolality in both individuals with impaired concentrating capacity and individuals

with intact concentrating capacity, we presume that the established large inter- and intraindividual variability indicates that urine aquaporin-2 level is not a good marker of activation of the vasopressin 2 receptor–cyclic AMP–aquaporin-2-urine osmolality cascade, in conflict to what has been suggested previously in other studies (Elliot et al., 1996; Ishikawa et al., 1998; Kanno et al., 1995; Rai et al., 1997). Based on our results it is therefore not possible to entirely exclude a direct involvement of aquaporin-2 (by means of production phosphorylation release or insertion in the apical membrane). There are certain limitations to our study. We did not include a healthy control group. Including a healthy control group in our experiment could have revealed important information on actual differences in the urinary cyclic AMP and aquaporin-2 levels, in response to water loading and administration of 1-desamino-8-D-arginine-vasopressin between those not exposed to long-term lithium treatment and those under long-term lithium treatment not suffering from a kidney urine concentrating deficit and those under long-term lithium treatment suffering from a kidney urine concentrating deficit. We only followed patients for 3.5 h post 1-desamino-8-D-arginine-vasopressin administration. Possibly a follow-up period of 3.5 h is insufficient to assure full recovery of the medullary hyperosmolal status, which could have been diminished due to water loading. We were not able to discuss the influence of concomitantly used medication, due to the relatively small population size. Further research is warranted to investigate the effects of concomitant use of medication on the occurrence of lithium-induced NDI.

In conclusion, our findings are the first indication that NDI induced by long-term lithium use in man is caused by a mechanism leading to inadequate production of cyclic AMP, thus implicating that the mechanism of lithium-induced kidney urine concentrating deficits is to be found at the vasopressin 2 receptor or within the vasopressin 2 receptor–cyclic AMP part, rather than in the cyclic AMP–aquaporin-2 part of the kidney urine concentrating cascade. It remains to be elucidated, which specific part of the vasopressin 2 receptor–cyclic AMP cascade (for instance G-proteins or adenylate cyclase e.g.) or perhaps the principal cells in general, are the major targets for renal lithium toxicity in man.

Additionally, variability, as reported in literature, in individual susceptibility for renal lithium toxicity suggesting involvement of a genetic factor resulting in predisposition, remains the subject for future investigation.

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