Renal handling of lithium and the effects of mannitol and arginine vasopressin in man

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1. A study has been undertaken in six healthy male subjects to clarify whether post-proximal segments of the nephron contribute to the renal handling of lithium under conditions of maximal forced osmotic load diuresis and arginine vasopressin-induced antidiuresis. Increments in the fractional clearance of free water, as a measure of effect at the proximal tubule, were positively correlated with incremental changes in flow rate, factored for glomerular filtration rate (mean \( r = 0.80 \pm 0.12, P < 0.001 \)), and fractional excretion of lithium (mean \( r = 0.84 \pm 0.06, P < 0.001 \)). Changes in flow rate and fractional excretion of lithium were also closely correlated with one another (mean \( r = 0.81 \pm 0.06, P < 0.001 \)), and the mean slope of these regression lines was not significantly different from unity (1.18; 95% confidence interval 0.76–1.59). These results show that, under conditions of maximal hydration, mannitol-induced changes in proximal tubular function were closely correlated with induced changes in the fractional excretion of lithium.

2. Infusion of arginine vasopressin alone (0.5 m-units/min) caused a marked reduction in both fractional clearance of free water (10.7% ± 1.2% to −1.2% ± 0.2%, \( P < 0.001 \)) and flow rate factored for glomerular filtration rate (14.0 ± 1.5 to 8.0 ± 0.2%; \( P < 0.001 \)) while the fractional excretion of lithium showed only a small non-significant decrease (25.3% ± 2.0% to 23.3% ± 2.2%). A similar dissociation was noted between fluid and lithium excretion when arginine vasopressin was superimposed on mannitol infusion with reductions in the fractional clearance of free water (12.7% ± 1.0% to −0.9% ± 0.7%, \( P < 0.001 \)) and flow rate (18.6% ± 1.5% to 5.7% ± 1.0%; \( P < 0.001 \)), while the fractional excretion of lithium showed a significant increase (28.4% ± 1.7% to 33.1% ± 2.4%; \( P < 0.05 \)). The lack of correlation between fluid and lithium excretion, in the presence of arginine vasopressin with or without mannitol, indicates that the late distal tubule and collecting duct have little or no significant capacity to reabsorb lithium.

3. These findings, taken as a whole, strengthen the view that renal tubular handling of lithium is primarily a proximal event.

INTRODUCTION

The fractional excretion of lithium (\( \text{FE}_{\text{Li}} \)) has been proposed as an index of fluid delivery out of the proximal renal tubule, both in the intact kidney and in micropuncture studies [1–3]. Other indirect markers for assessing effects on proximal tubular function, such as maximal flow rate, and urinary excretions of free water, urate and phosphate, have also been used in the past. These, however, suffer from several drawbacks as they may all be influenced by the hormonal or metabolic state of the individuals at the time of assessment [4, 5]. The availability of a reliable index, such as lithium clearance, which is relatively simple and easily applicable to either clinical or research settings may provide useful insight into various transport processes in the kidney involved in maintaining a normal homeostatic state. In addition, any abnormalities in the renal handling of salt and water which contribute to the genesis of, or exacerbate, disease states such as hypertension, congestive cardiac failure and chronic renal failure may be localized and evaluated using lithium clearance techniques.

The validity of this clearance technique is based on a number of underlying premises, which are: (a) lithium is filtered only at the glomerulus and is not secreted into the tubular lumen in any segment of the nephron (Fig. 1a); (b) lithium reabsorption is localized exclusively in the proximal segment of the nephron; and (c) lithium reabsorption parallels the reabsorption of water (and/or sodium) such that the tubular concentration of lithium (\( \text{TF}_{\text{Li}} \)) remains constant and equal to the plasma concentration (\( \text{P}_{\text{Li}} \)) throughout the length of the proximal tubule under a variety of experimental conditions.

While a number of studies have supported most

Key words: arginine vasopressin antidiuresis, distal tubule, hydration, lithium clearance, mannitol diuresis, proximal tubule, sodium clearance.

Abbreviations: AVP, arginine vasopressin; FE, fractional excretion; GFR, glomerular filtration rate; IOD, iodohippurate; RPF, renal plasma flow; TAL, thick ascending limb; TF, tubular fluid.

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of these underlying assumptions [1, 2, 6–9], questions have been raised about using lithium clearance as a marker for fluid delivery out of the proximal tubule, both in general [10] and under certain experimental states such as salt or volume depletion, haemorrhagic hypotension or following treatment with non-steroidal anti-inflammatory agents [11–14]. Recent studies in animals and in man have also highlighted a possible role for the loop of Henle (Fig. 1b) and the more distal segments of the nephron as potential sites of lithium reabsorption. As much as 15% of the filtered load of lithium (Fig. 1) has been reported to be reabsorbed in the loop of Henle [13, 15, 16]. If indeed such a significant fraction of lithium is reabsorbed in the loop of Henle, then any change in the delivery out of the proximal tubule, as measured by renal clearance of lithium, is likely to be a considerable underestimate of the true value. The proximal tubule is responsible for the bulk of reabsorption of both solutes and water from the glomerular filtrate, and even small changes in its reabsorptive capacity may have a profound effect on salt and water homeostasis. If this were to be the case, then lithium clearance would not be a reliable index of proximal tubular function and any inference derived from such data can only be of limited use.

Changes in proximal tubular handling of fluid and electrolytes can be achieved by using either carbonic anhydrase inhibitors or non-reabsorbable solutes, such as mannitol [17]. Carbonic anhydrase inhibitors can result in metabolic acidosis, which can have an additional impact on renal handling of lithium [18, 19]. Mannitol, on the other hand, does not cause acid–base disturbances but is known to inhibit transport processes beyond the proximal tubule when administered in large doses [20–22]. Whether such post-proximal effects of mannitol also occur at lower doses is not known. In addition, net proximal reabsorption of lithium has also been shown to follow closely reabsorption of water under conditions of mannitol-induced osmotic diuresis [9]. If it can be demonstrated that changes induced in the proximal tubule result in a commensurate change in lithium excretion, then the lithium clearance technique can be used as a reliable index of delivery out of the proximal tubule. The purpose of the present study was to investigate (a) whether the effects of low-dose infusion of mannitol are confined to the proximal tubule; (b) how mannitol-induced changes in proximal handling of fluid and electrolytes may alter renal handling of lithium in the post-proximal segments of the nephron; and (c) whether changes in distal water flux, induced by the known hydro-osmotic action of arginine vasopressin (AVP) on the distal nephron and collecting ducts, contributes significantly to changes in the renal handling of lithium.

Part of this work was presented at XX Nordic Congress of Physiology and Pharmacology, Copenhagen, August 1992 [23].

METHODS

Six healthy male volunteers (mean age 29 years; range 24–38) gave their informed consent before undergoing full physical examination and biochemical and haematological screening. The study protocol was approved by the Riverside Health District Ethics Committee and fully complied with the Helsinki Declaration. Subjects refrained from smoking and consuming alcohol or foods with a high salt content or containing xanthine for 3 days before and on study days. All subjects fasted from 22.00 hours, after ingesting a single 400-mg dose of lithium carbonate (Priadel; Delandale Laboratories, Canterbury, Kent, U.K.), on the evening preceding each study day. This single dose of lithium carbonate, administered 12 h previously, does not modify the basal renal state under conditions of sustained
water diuresis [24]. Subjects were investigated on two separate occasions with a minimum of 1 week, and a maximum of 8 weeks, between the two investigations. After a light breakfast, subjects were orally hydrated with drinking water (20 ml/kg body weight) over a 30-min period. An intravenous line was established in one forearm, through which a bolus dose of $^{51}$Cr-labelled EDTA ($^{51}$CrEDTA; 25 $\mu$Ci) and $^{125}$Iiodohippurate ($^{125}$IIOH; 7.5 $\mu$Ci) was administered. This was followed by a sustained infusion of both radioisotopes at a rate of 2 ml/min (75 $\mu$Ci of $^{51}$CrEDTA and 22.5 $\mu$Ci of $^{125}$IIOH in 1000 ml of 4 g/l D-glucose with 1.8 g/l NaCl solution). Blood samples were drawn, via an indwelling intravenous cannula in the opposite arm, at half-hourly intervals. Urine was collected, by spontaneous voiding, at 20-min intervals. In order to maintain a state of maximal hydration, water was drunk in amounts equal to the volume of urine collected. When urine flow had exceeded 10 ml/min, and both the flow rate and urine osmolality had stabilized for three consecutive 20-min intervals, subjects were randomly assigned to be infused with either mannitol (sequence 1) or AVP (sequence 2) in order to induce osmotic diuresis or antidiuresis. In sequence 1, mannitol (50 g/l with 6 g/l NaCl) was infused at a fixed rate of 4 ml/min and urine collections continued at 20-min intervals. At 140 min after the start of mannitol infusion, AVP (Pitressin, Parke Davis; 0.50 m-units/min) was co-infused with mannitol for a further 120 min. Subjects were advised to carry on collecting urine samples at 20-min intervals and, when this was not possible, the collection interval was extended to 40 or 60 min. In sequence 2, a sustained antidiuresis was achieved by infusing AVP (0.50 m-units/min = 1.16 pmol/min) for a period of 120–240 min after the establishment of steady-state maximal hydration. Urine was collected over the shortest period possible, which ranged from 20 min to 120 min, during sustained antidiuresis. Mannitol (50 g/l with 6 g/l NaCl) was subsequently co-infused (4 ml/min) for a further 120 min and urine collections continued in the manner described above. Investigations were repeated after a minimum interval of 1 and a maximum of 8 weeks, with the order of infusions reversed on the second occasion.

Mannitol was dissolved in 0.6% saline solution in order to deliver sufficient amounts of sodium to counter both the basal and mannitol-induced salt losses and therefore maintain the subjects in a salt-balanced state. The mean urinary excretion of sodium, from the start to the end of ‘mannitol alone’ infusion, amounted to 54 ± 4 mmol and was not significantly different from the total amount of salt (60 mmol) delivered intravenously as D-glucose saline and the mannitol infusion. The subjects were therefore maintained in a salt-neutral state, and thus any adverse impact on the renal handling of sodium or of lithium which could have resulted from mannitol-induced saluresis was minimized.

**Laboratory methods**

$^{51}$CrEDTA and $^{125}$IIOH activities were determined simultaneously in 1-ml aliquots of urine and plasma samples using a gamma-counter (Canberra Packard model 5550, Pangbourne, Berkshire, U.K.). Plasma and urinary lithium analyses were carried out using an atomic absorption spectrophotometer (Perkin-Elmer model 603, Beaconsfield, U.K.). All other plasma and urinary analyses were carried out as described previously [25]. Mannitol is known to interfere with chemical analysis of phosphate, and, in order to eliminate this interference, all urine samples were reanalysed after successive serial dilutions until a constant phosphate value was obtained [26].

**Calculations and statistics**

Renal clearances and fractional excretions were calculated using standard formulae:

$$C_l = U \times V / P$$ and $$FE(\%) = (C_l / GFR) \times 100$$

where $C_l =$ renal clearance, $U =$ urine concentration, $V =$ flow rate and $P =$ plasma concentration. Glomerular filtration rate (GFR) was estimated as renal clearance of $^{51}$CrEDTA, while renal plasma flow (RPF) was calculated as the clearance of $^{125}$IIOH. Free water clearance ($C_{H2O}$) was calculated from $C_{H2O} = V -$ osmolar clearance ($C_{osm}$), while free water reabsorbed was calculated as $T_{H2O} = C_{osm} - V$.

Group data were expressed as means ± SEM, with each subject acting as his own control. Changes in renal parameters were monitored over the entire period of infusion in order to analyse time trends as described by Wallenstein et al. [27]. When assessing the overall response, an average of the last two consecutive baseline collection periods (–40 to 0 min), just before infusion of mannitol or AVP alone served as control values. The mean of the last two periods, when on mannitol alone, AVP alone or when AVP was co-infused with mannitol, was taken as the respective test responses (i.e 100–140 min period for mannitol alone and 220–260 min for mannitol plus AVP). In all cases data were analysed using analysis of variance for repeated measures before applying Newman–Keuls test and changes were considered to be significant at the $P < 0.05$ level.

**RESULTS**

Individual baseline values for urine flow rate ($V$), glomerular filtration rate (GFR), renal plasma flow (RPF), urine osmolality ($U_{osm}$), flow rate factored for GFR ($V$/GFR), fractional excretions of free water (FE $C_{H2O}$), osmolytes (FEosm), phosphate (FEpo4), sodium (FENa), potassium (FEK) and lithium (FELi) reached a steady state between 80 and 120 min after the initial oral water. The basal values
before the commencement of mannitol infusion are shown in Table 1. A mean flow rate of $15 \pm 1 \text{ ml/min}$ was achieved at a low urinary osmolality of $63 \pm 4 \text{ mosmol/kg H_2O}$. An atypically low $\text{FE}_{\text{PO}_4}$ (3.3%) occurred in one subject (no. 1), though the renal excretory pattern was normal in all other aspects. The equivalent baseline values for sequence 2, obtained before AVP infusion, were not significantly different from those seen in sequence 1 (Table 1). The atypically low $\text{FE}_{\text{PO}_4}$ (3.8%) was again seen in subject no. 1 (Table 1).

**Sequence I: lithium clearance and mannitol infusion**

(Table 2, Figs. 2 and 3)

During the period of mannitol infusion there was little change in renal haemodynamics, except for a small, but significant, drop in GFR. This decrease in GFR persisted throughout the experiment and ranged from $-2$ to $-9 \text{ ml/min}$ (mean change $= -6 \pm 1$; $P < 0.01$) at 100–140 min from the start of mannitol infusion (Table 2, Fig. 2). There were significant time-trends towards increases in $V/GFR$ ($P < 0.01$), $\text{FE}_{\text{H}_2\text{O}}$ ($P < 0.05$), $\text{FE}_{\text{sm}}$ ($P < 0.01$; not illustrated), $\text{FE}_{\text{K}}$ ($P < 0.05$) and $\text{FE}_{\text{Li}}$ ($P < 0.01$; Figs. 2 and 3). The positive time-trend for $\text{FE}_{\text{PO}_4}$ (Fig. 3; $P < 0.06$) failed to reach statistical significance owing to inter-individual variations. There was a significant downward trend for $\text{FE}_{\text{K}}$ ($P < 0.01$; Fig. 3), while $\text{FE}_{\text{sm}}$ remained unchanged (Table 3). Net overall changes in these measurements, represented by means of individual excretions at 100–140 min when compared with control (−40 to 0 min), are summarized in Tables 2 and 3.

During mannitol infusion a positive correlation was observed, in each subject, when individual increments ($\Delta$) in $\text{FE}_{\text{H}_2\text{O}}$, i.e. net changes in each 20-min period over corresponding baseline values, were compared with similar increments in $V/GFR$ (mean $r = 0.80 \pm 0.12$, $P < 0.001$; Fig. 4) or $\text{FE}_{\text{Li}}$ (mean $r = 0.84 \pm 0.06$, $P < 0.001$; Fig. 4). Similarly, a

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**Table 1. Weight and basal renal function in six healthy subjects undergoing maximal water diuresis.** Each baseline value represents a mean of two consecutive 20-min periods following establishment of steady-state maximal hydration before infusion of mannitol (sequence 1). The corresponding baseline values before AVP infusion (sequence 2) are shown in parentheses.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>70 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>73 ± 3</td>
</tr>
</tbody>
</table>

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**Table 2. Effect of infusion of mannitol alone and mannitol co-infused with AVP on renal function following establishment of steady-state maximal water diuresis.** Zero time represents the start of mannitol infusion (see methods section for more details). Abbreviation: FF, filtration fraction. Statistical significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control; †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with infusion of mannitol alone.

<table>
<thead>
<tr>
<th></th>
<th>Control (−40 to 0 min)</th>
<th>Mannitol (100–140 min)</th>
<th>Mannitol + AVP (220–260 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>104 ± 7</td>
<td>99 ± 6**</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>434 ± 28</td>
<td>427 ± 26</td>
<td>461 ± 29</td>
</tr>
<tr>
<td>FF (%)</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>$U_{\text{osm}}$ (%)</td>
<td>63 ± 4</td>
<td>91 ± 2***</td>
<td>385 ± 54****††††††††</td>
</tr>
<tr>
<td>$V/GFR$ (%)</td>
<td>14.5 ± 1.3</td>
<td>18.6 ± 1.5**</td>
<td>5.7 ± 1.0**†††††††††††</td>
</tr>
<tr>
<td>$\text{FE}_{\text{H}_2\text{O}}$ (%)</td>
<td>11.4 ± 1.3</td>
<td>12.7 ± 1.0</td>
<td>−0.9 ± 0.7**†††††††††††</td>
</tr>
<tr>
<td>$\text{FE}_{\text{sm}}$ (%)</td>
<td>3.2 ± 0.3</td>
<td>5.9 ± 0.6***</td>
<td>6.8 ± 0.6***</td>
</tr>
<tr>
<td>$\text{FE}_{\text{K}}$ (%)</td>
<td>9.9 ± 1.7</td>
<td>14.1 ± 2.8</td>
<td>11.9 ± 3.4</td>
</tr>
<tr>
<td>$\text{FE}_{\text{Li}}$ (%)</td>
<td>1.3 ± 0.2</td>
<td>1.9 ± 0.3†††††††††††††††</td>
<td>2.1 ± 0.4**‡‡‡‡‡‡‡‡‡‡‡</td>
</tr>
<tr>
<td>$\text{FE}_{\text{PO}_4}$ (%)</td>
<td>20.3 ± 2.1</td>
<td>22.9 ± 2.1***</td>
<td>10.5 ± 1.6**‡‡‡‡‡‡‡‡‡‡‡</td>
</tr>
<tr>
<td>$\text{FE}_{\text{Li}}$ (%)</td>
<td>24.3 ± 1.8</td>
<td>28.4 ± 1.7*</td>
<td>33.1 ± 2.4**†††††††††††</td>
</tr>
</tbody>
</table>
Mannitol, arginine vasopressin and lithium clearance

Fig. 2. Time course of effect of infusion of mannitol alone and mannitol with AVP on glomerular filtration rate (GFR, right axis), fractional excretion of lithium (FE\textsubscript{Li}%), and flow rate factored for GFR (V/GFR\% left axis). The arrows indicate the start of mannitol infusion (at zero time) and the addition of AVP to the infusate (at 140 min after the start of mannitol infusion).

Table 3. Effect of infusion of mannitol alone, mannitol coinfused with AVP (sequence 1), AVP alone and AVP coinfused with mannitol (sequence 2) on fractional excretion of calcium (FE\textsubscript{Ca}%) and magnesium (FE\textsubscript{Mg}%) after establishment of steady-state maximal -water diuresis (control). Statistical significance: *P < 0.05 compared with control. †P < 0.05 compared with infusion of mannitol alone.

<table>
<thead>
<tr>
<th>Sequence 1</th>
<th>FE\textsubscript{Ca} (%)</th>
<th>FE\textsubscript{Mg} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5 ± 0.4</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>Mannitol</td>
<td>1.7 ± 0.3</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>Mannitol + AVP</td>
<td>2.6 ± 0.5†</td>
<td>4.6 ± 0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sequence 2</th>
<th>FE\textsubscript{Ca} (%)</th>
<th>FE\textsubscript{Mg} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.1 ± 0.4</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>AVP</td>
<td>1.9 ± 0.4</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>AVP + mannitol</td>
<td>2.0 ± 0.6</td>
<td>3.9 ± 0.9</td>
</tr>
</tbody>
</table>

A linear association was also obtained when the changes in ΔFE\textsubscript{Li} were compared with ΔV/GFR (mean r = 0.81 ± 0.06, P < 0.001; Fig. 5). The mean of all the slopes of the individual regression lines (1.18; 95% confidence interval = 0.86–1.59), though above 1, was not significantly different from the line of identity (Fig. 5), while the mean of the intercept (−0.62 ± 0.41) was not different from zero.

Lithium clearance and mannitol co-infused with AVP (Table 2 and Figs. 2 and 3)

When AVP was superimposed on mannitol infusion, there was no further change in GFR (Fig. 2).
However, addition of AVP to the infusion produced a prompt and a significant decrease in both V/GFR (Fig. 2; \( P < 0.01 \)) and FE \( \text{CH}_{2} \) (\( P < 0.001 \); Table 2). The overall rate of change of \( \text{FE}_{\text{Li}} \) continued to rise more steeply during the co-infusion of AVP than with mannitol alone (\( 0.05 \pm 0.01 \) versus \( 0.03 \pm 0.01 \) \( \text{FE} / \text{min} \); \( P < 0.05 \), Fig. 3). \( \text{FE}_{\text{Na}} \) also showed a similar rise in four consecutive 20-min periods before reaching a peak of \( 2.3 \pm 0.5 \) (\( P < 0.01 \)) at 200–220 min from the start of mannitol infusion (60–80 min period following start of AVP infusion; Fig. 3). Beyond this period, \( \text{FE}_{\text{Na}} \) declined for the next two collections, with the final value of \( 2.1 \pm 0.4 \) still remaining significantly greater than premannitol control (\( 1.2 \pm 0.2 \); \( P < 0.05 \)). The significant downward trend in \( \Delta \text{FE}_{\text{K}} \) seen with mannitol alone continued during the co-infusion of mannitol with AVP, though at a much reduced rate (Fig. 3). The final \( \Delta \text{FE}_{\text{K}} \) value of \( 10.5 \pm 1.6 \) was significantly different from control (\( 20.3 \pm 2.1 \); \( P < 0.001 \)) but not from \( \Delta \text{FE}_{\text{K}} \) that had been attained at the end of infusion with mannitol alone (\( 12.9 \pm 2.1 \); not significant; Table 2 and Fig. 3). The rise in \( \Delta \text{FE}_{\text{PO}_{4}} \) seen with mannitol alone was reversed during the period of AVP co-infusion such that \( \Delta \text{FE}_{\text{PO}_{4}} \) declined, at a rate parallel to \( \Delta \text{FE}_{\text{K}} \), for the remainder of the study (Fig. 3). A significant increase, over control value, was seen in \( \text{FE}_{\text{Ca}} \) (\( P < 0.05 \); Table 3) and, though \( \text{FE}_{\text{Mg}} \) also increased by a similar amount, the final value was not significantly different from control or mannitol alone period.

**Sequence 2: lithium clearance with AVP alone followed by AVP co-infused with mannitol**

The effect of AVP infusion on \( \text{FE}_{\text{Li}} \) paralleled that of \( \text{FE}_{\text{Na}} \) (Fig. 6). \( \text{FE}_{\text{Li}} \) declined in four, remained unchanged in one and increased in the remaining subject at the end of the AVP infusion (25.3 ± 2.0 versus 23.3 ± 2.2; \( P < 0.10 \), not significant). There was a significant rise in \( \text{FE}_{\text{Li}} \) from 23.3 ± 2.2 to 28.1 ± 3.0 (\( P < 0.05 \)) when AVP was co-infused with mannitol (Fig. 6). This latter value (28 ± 3) was not significantly different from the control (25.3 ± 2.0; not significant). AVP alone caused a similar decline in \( \text{FE}_{\text{Na}} \) in the same five subjects (mean \( \text{FE}_{\text{Na}} \) 1.3 ± 0.4 versus 1.1 ± 0.3; not significant) and a recovery in \( \text{FE}_{\text{Na}} \) (1.3 ± 0.3; \( P < 0.05 \)) after co-infusion with mannitol (Fig. 6). Urinary osmolality rose from a mean basal value of (mosmol/kg \( \text{H}_{2}\text{O} \)) 64 ± 6 to 749 ± 49 (\( P < 0.001 \)) at the end of infusion with AVP alone. There were no significant changes in renal excretion of calcium and magnesium (Table 3). Co-infusion of AVP with mannitol, for a further 120 min, led to a small but significant decrease in the ability of the kidney to concentrate urine; urinary osmolality fell to 626 ± 39 (\( P < 0.01 \); Fig. 7). There were non-significant changes in \( \text{FE}_{\text{PO}_{4}} \), which increased from 9.9 ± 2.4% (control) to 11.7 ± 1.4% (AVP alone; not signifi-
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Fig. 7. Effect of infusion of AVP alone followed by AVP co-infused with mannitol on (a) urinary osmolality (Δ) and (b) fractional free water reabsorbed (F) in each of the six subjects studied. The means and SEM are shown as closed symbols. Control data (pre-AVP infusion) have been excluded from this Figure for reasons of clarity. Statistical significance: *P<0.01, **P<0.001 compared with AVP alone.

DICUSION

The proximal and post-proximal actions of the osmotic diuretic, mannitol, have been investigated using micropuncture techniques. Less than half of the total diuretic effect of mannitol can be accounted for by inhibition of sodium and water transport in the proximal tubule, with the thick ascending limb (TAL) of the loop of Henle contributing in a major way to the overall diuresis [28]. While such an effect may occur with mannitol solutions of very high tonicity (10–20% solutions equal approximately 600–1200 mosmol/kg H₂O) used clinically or experimentally, this may not necessarily be true when nearly isotonic solutions of mannitol (5% solution equals approximately 300–400 mosmol/kg H₂O) are infused. It is therefore possible that, at low doses, mannitol may have a diuretic profile in which the contribution of proximal and post-proximal segments of the nephron may differ from those reported using higher doses [28].

In the present study we have demonstrated that mannitol infusion, at low doses, did not affect the post-proximal segments of the nephron during the first 140 mins of infusion. There was a progressive increase in FE Ca, accompanied by parallel increases in V/GFR and FEPO₄, while renal excretions of calcium and magnesium remained unaltered. Taken together, such changes support the view that the primary inhibitory locus of action of low-dose mannitol resides within, but not beyond, the proximal tubule. Similar evidence has been used to delineate proximal tubular actions of other renally active agents such as diuretics [5]. The possibility that some of the actions of mannitol may also be mediated indirectly, secondary to release of atrial natriuretic peptides, should also be considered [29] though their contribution would be expected to be small [30]. Clearly, from our findings, we can be confident in using low-dose infusion of mannitol to evoke selective changes in the renal handling of fluid and solutes within the proximal tubule.

Changes in renal haemodynamics, with a small decrease in GFR in the absence of significant changes in RPF, were consistent with differential effects of mannitol on cortical and medullary nephrons [31]. It is likely that increase in intratubular osmotic pressure as well as dilatation of efferent arterioles, following mannitol infusion, may also contribute to the observed effects on GFR [32].

Infusion of mannitol produced a paradoxical reduction in FE K. It has been demonstrated that, in water-loaded states, urinary excretion of potassium remains unchanged or shows a modest decline without altering plasma aldosterone [33, 34]. Data from micropuncture experiments generally show that excretion of potassium is a flow-dependent process such that any increase in tubular flow rate is associated with increased excretion of potassium, while a decrease in tubular flow results in increased reabsorption of potassium within the distal nephron [35]. Any natriuresis should also result in an increase in potassium excretion as the distal tubule compensates for increased salt delivery. The increase in the rate of reabsorption of potassium following diuresis induced by mannitol was therefore opposite to that expected. This suggests that mannitol could have activated a potassium-sparing mechanism, thus offsetting the effects of increased fluid and solute delivery which would otherwise favour potassium loss. How this is achieved remains unknown, but it could involve subtle alterations in aldosterone secretion, secondary to mannitol-induced expansion of extracellular volume [20].

By contrast with the changes observed in FE K, FEPO₄ increased after mannitol infusion. This trend was, however, reversed in the presence of AVP,
although AVP alone failed to have any effect on phosphate excretion. The possibility that the anti-phosphaturic effect of AVP, in the presence of mannitol, could have been an artifact due to increased interference by mannitol in the more concentrated urine with the assay of phosphate can be ruled out as necessary precautions were taken when analysing these sample [26]. The exact mechanism by which AVP reversed the phosphaturic effect of mannitol in sequence 1 of the study and why such an effect was not seen after mannitol was co-infused with AVP in sequence 2 remain to be elucidated. It is, however, clear that, after AVP-induced anti-diuresis, renal handling of phosphate becomes dissociated from that of lithium, and under such conditions FE\textsubscript{PO\textsubscript{4}} cannot be relied upon as an adequate marker for proximal events. This adds to our earlier observations, which also showed that the renal handling of phosphate is dissociated from renal handling of sodium during the post-diuretic retention phase [4].

The rate of rise of FE\textsubscript{Li}, in the presence of co-infusion of mannitol and AVP, was greater than that seen with mannitol alone. This change in the steepness of the slope probably reflects homeostatic compensation by the kidney after volume expansion induced by AVP infusion. The intensity and rapidity of the antidiuretic effect of AVP, seen in Fig. 2, must inevitably lead to expansion of extracellular fluid volume and subsequent activation of homeostatic mechanisms, such as increased sodium and water excretion [36–38]. This was clearly seen in the present study, in which FE\textsubscript{Na} rose to reach a peak before declining after the addition of AVP to mannitol infusion. This late decline in FE\textsubscript{Na} probably reflects an increased ability of nephrons to reabsorb sodium in the presence of AVP [39, 40]. FE\textsubscript{Li} on the other hand, continued to increase throughout the period of infusion, thus dissociating the renal handling of lithium from that of sodium. The apparent dissociation of sodium absorption from lithium excretion in the final two periods of the investigation in sequence 1 casts doubt on the ability of AVP to promote lithium reabsorption under these conditions. The rise in FE\textsubscript{Li}, which was seen despite an extensive movement of fluid from the tubular lumen into the interstitium, also indicates that lithium transport across the distal and collecting duct epithelia, if present, cannot be attributed to solvent drag. The trend in the parallel decrease in FE\textsubscript{Li} and FE\textsubscript{Na}, after the infusion of AVP alone, was not consistent enough to define a specific reabsorption site in the nephron. Our results tend to support the view that AVP, at the doses used in this study, does not have a significant impact on renal clearance of lithium.

The power of the study was such that the minimum detectable differences in sequence 1 [$\alpha=0.05; \beta=0.10; v_2$ (degrees of freedom)=10] were 6.48% of the filtered load of lithium and 0.82% of the filtered load of sodium. The corresponding values for sequence 2 were 4.61% (lithium) and 0.59% (sodium). It remains to be determined whether the observed decrease in FE\textsubscript{Li} and FE\textsubscript{Na} would have been significant had a larger number of subjects been used or, alternatively, if the infusion rate of AVP had been different. If such an effect were to be confirmed, then the AVP-mediated lithium-retaining action must reside in a nephron segment other than the TAL, as the human loop of Henle, unlike that of the mouse, is devoid of vasopressin receptors [41].

Accurate changes in the delivery of solute or fluid out of the proximal tubule can only be measured directly by micropuncture, though allowances have to be made for reabsorption which may occur in the pars recta [42, 43]. Such invasive approaches cannot be applied to study of the human proximal tubule in vivo, and so one becomes dependent on indirect methods for estimating delivery out of this nephron segment. V/GFR, under conditions of maximal water diuresis, and phosphate excretion have been used as the simple indices of delivery out of the proximal tubule. Both these parameters suffer from drawbacks as they fail to take into account any AVP-independent back-diffusion of free water along the post-proximal segments of the nephron [28, 44, 45] or, in the case of phosphate, the effects of parathyroid hormone and disparity between increased reabsorption of sodium in the proximal tubule coupled with simultaneous increase in urinary excretion of phosphate [4]. This dissociation in renal handling of sodium and phosphate has again been confirmed in this study.

Although the absolute value of V/GFR, by itself, may not be a suitable measure of proximal tubular function, relative changes in V/GFR may still prove useful provided there are minimal changes in factors that could influence post-proximal segments. In the present study, a small change in the corticomedullary gradient was observed after mannitol over a 2-h period, the concentrating capacity of the kidney being reduced such that mean urine osmolality fell from 749 (AVP alone) to 626 (AVP+mannitol; $P<0.01$). This was to be expected, as mannitol does cause some increase in medullary renal blood flow [31] which contributes to medullary washout. While this may be true for short exposure to mannitol (i.e. ≤140 min) this may not necessarily be so if mannitol were to be infused for prolonged periods or if higher dosages were given. The impact of the small alterations in the corticomedullary gradient seen in this study is likely to be negligible, thereby allowing us to interpret the change seen in the generation of free water as purely a proximal event. The parallel changes in $\Delta$FE C\textsubscript{HCO\textsubscript{3}} and $\Delta$V/GFR, and the distinct lack of an early effect on the renal excretion of calcium and magnesium, add further support to the view that mannitol had no effect on the loop of Henle.

The index of proximal delivery that is held to be more reliable than phosphate is the renal clearance of lithium, though there have been recent sugges-
tions that this may also underestimate proximal tubular delivery out of this segment [46] (Fig. 1). If it is assumed that lithium is not reabsorbed in any of the post-proximal segments of the nephron (Fig. 1) and that the $T_{\text{Fr}}/P_{\text{Fr}}$ ratio in the proximal tubule approximates unity, then a significant degree of correlation would be expected between the relative changes in $V/GFR$ and $F_E_{\text{Li}}$ provided the post-proximal function remained unaltered. Alternatively, if lithium were handled in the post-proximal segments of the nephron (Fig. 1), by either active or voltage-dependent processes [40], then the experimentally derived slope of the relationship between $\Delta V/GFR$ and $\Delta F_E_{\text{Li}}$ would differ from unity. When $\Delta V/GFR$ was plotted against $\Delta F_E_{\text{Li}}$ after the infusion with mannitol alone, a linear relationship was significantly different from unity. It is interesting to note that the value of 1.18 obtained in the present in vivo study is very close to the consensus view of the $T_{\text{Fr}}/P_{\text{Fr}}$ ratio of 1.10 (range 1.06–1.16) obtained in micropuncture experiments involving proximal tubules [42]. The observation that increases in $F_E_{\text{Li}}$ paralleled the changes in FE $C_{\text{H}_2\text{O}}$ (Fig. 4) lends additional support to the view that there was no significant degree of lithium reabsorption in or beyond the loop of Henle.

In conclusion, low-dose mannitol acts predominantly in the proximal tubule and leads to a significant increase in delivery of fluid and electrolyte out of this segment. The close relationship between relative changes in $V/C_{\text{H}_2\text{O}}$, $F_E_{\text{Li}}$ and $F_E_{\text{PO}_4}$ indicates a lack of significant reabsorption of lithium in the nephron segments beyond the proximal tubule. In addition, data from AVP infusion experiments do not support significant distal reabsorption of lithium via solvent drag. The presence of a minor and as yet undefined lithium-retaining site of action for AVP, however, cannot be completely ruled out. Changes in $F_E_{\text{Li}}$ remain a reliable marker for the in vivo estimation of fluid delivery out of the proximal tubule under conditions of mannitol-induced diuresis. Caution, however, must be exercised when interpreting $F_E_{\text{PO}_4}$ data or $F_E_{\text{Li}}$ data in the present of AVP-induced antidiuresis.

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REFERENCES


