Hydration status affects sodium, potassium, and chloride transport across rat urothelia

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Spector DA, Deng J, Stewart KJ. Hydration status affects sodium, potassium, and chloride transport across rat urothelia. Am J Physiol Renal Physiol 305: F1669–F1679, 2013. First published October 1, 2013; doi:10.1152/ajprenal.00353.2013.—Recent data suggest possible net transport of urinary constituents across mammalian urinary tract epithelia (urothelia). To evaluate the effect of animal hydration status on such transport, we instilled urine collected during 2-day water deprivation, water loading, or ad libitum intake into isolated in situ bladder(s) of groups of rats undergoing one of the same three hydration states. After 1-h bladder dwell, we retrieved the urine and measured differences in volume and solute concentrations between instilled and retrieved urine. We previously reported results regarding changes in urine volume and net urea and creatinine transport and herein report the results of net urinary sodium, potassium, and chloride transport in the same animals. During water-loading conditions, urinary concentrations of Na, K, and Cl rose 4.9 (30.7%), 2.6 (16.5%), and 6.0 meq/l (26.8%), respectively, indicating urothelial reabsorption of each ion. During ad libitum intake, urinary K and Cl concentrations fell 33.6 (14.8%) and 28.4 meq/l (12%), respectively (Na did not change), and during water deprivation urine Na, K, and Cl concentrations fell dramatically by 53.2 (18.6%), 159.4 (34.6%) and 133.7 meq/l (33.8%), respectively, reflecting urothelial reabsorption of each ion. For each ionic species, two factors independently influenced transport: instilled urinary ion concentration and animal hydration state. These results demonstrate significant regulated ion transport across mammalian urothelia, support the notion that lower urinary tract modifies final urine, and suggest that the lower urinary tract may play a role in local and whole animal solute homeostasis.

Although the main function of the lower urinary tract in mammals is to provide for transport and storage of urine produced by the kidney (12), there is increasing evidence that mammalian urothelia (the epithelial cell lining of the urinary tract from the renal pelvis to the proximal urethra) is a very complex tissue with numerous unexpected functions (reviewed in Refs. 3, 21, and 24). Recent work indicates that one such function might be to alter the concentration of urinary constituents as a result of secretion of urothelial solutes into urine or the converse, reabsorption of urinary solutes across urothelium and into deeper bladder tissues, thereby suggesting a role in urinary tract tissue and/or whole body water and solute homeostasis (34, 35, 39, 43). Such transport is unexpected given the evidence from Ussing chamber studies that mammalian urothelia possess exceptionally low permeabilities to urinary constituents (5, 13, 17, 28). However, there is a long record of vectorial solute transport across urothelia (8, 11, 14, 15, 16, 19, 25, 26, 31, 23, 42). For example, using isolated bladder preparations in dogs, Rapoport and coworkers (31) and Hakim and coworkers (11) described net sodium and chloride and potassium (31) reabsorption from artificial solutions dwelling in the bladder across the urinary bladder epithelium. The direction of the flux in both studies was dependent on the analyte concentration in the instilled solution. Thus, at bladder fluid sodium and chloride concentrations near those in plasma, there was little or no net ion flux, but with higher concentrations there was increased ion reabsorption. Similar results for sodium and chloride transport were described in rats by Hohbrugger (15) who used an isolated bladder model in which the bladder was instilled with either hypertonic (700 mM NaCl) or hypotonic (70 mM NaCl) solutions. After 1 h, there was a rapid fall in bladder sodium concentration from the hypertonic solution, and a slight rise in sodium concentration in bladders of rats instilled with hypotonic solution. Interestingly, after bladder dwell urea was found in both solutions, indicating urea secretion from urothelia into bladder fluid. Although the significance of some of the early studies might be lessened due to the use of unphysiological conditions and/or models, two investigator groups utilizing exacting physiological conditions in vivo models demonstrated net vectorial transport across urothelia (23, 42). Thus Walser et al. (42) described small but significant urinary urea (7.1%), and potassium (3.4%), reabsorption and sodium secretion (9.2%) across the ureteral urothelium during brief (3 min) ureteral perfusion in rats receiving ad libitum water. Similarly, Levinsky and Berliner (23) described net reabsorption of 20% urea, and 15% potassium and secretion of up to 15% sodium and chloride in dogs whose ureters and bladders were slowly perfused with urine for 30 min. That net transport occurs as well in humans was recently suggested by Shaﬁk and coworkers (32), who showed that urine pH, osmolality, and sodium and potassium concentrations changed (sodium and potassium were reabsorbed) as urine passed from the renal pelvis through the bladder in moderately hydrated humans with renal disorders.

The mechanism(s) whereby solute transport across urothelia occurs and whether and how this transport is regulated are unclear. Since vectorial transport had only been described as occurring down a urine-blood concentration gradient, until recently it was assumed that any such transport must be accounted for by passive diffusion. However, the recent discovery in urothelial cells of multiple membrane channels and transporters, including those for water (36), sodium (25, 26, 33, 43), chloride (43), urea (27, 38, 39), and potassium (37, 40, 43, 46; reviewed in Ref. 21) has resulted in consideration of the
notion that urothelial channels and transporters may play an active role in net vectorial transport across mammalian urothelia and thereby in potentially regulated local urinary tract and whole animal water and solute homeostasis.

To study mammalian urothelial water and solute transport and building on models utilized in past studies, we recently developed and reported on an in vivo, in situ bladder model which closely mimics physiological conditions in rats (34, 35). To study the effect of animal hydration status on urothelial water and solute transport, we subjected groups of rats to one or a combination of water-deprivation, water-loading, or ad libitum water conditions and instilled urine obtained during various rat hydration states into the isolated in situ bladders of the same rats; after 1 h bladder dwell, we retrieved the urine and measured the differences between Instilled and Retrieved urine as regards urine volume and urine concentrations of urea, creatinine, sodium, chloride, and potassium. In a first publication on these experiments, we reported on the effect of whole animal hydration status on changes in urine volume and on concentrations and quantity of urine urea and creatinine (34).

We found that during bladder dwell that there was no change in urine volume (no net flux of water) for any hydration group but that there was significant transport of both urea and creatinine with direction and magnitude dependent on two factors related to hydration status: the instilled solute concentration and an independent bladder factor(s), resulting in increased solute reabsorption in the water-deprived state. In this second report on these experiments, we herein describe the effect of hydration status on urothelial transport of the major ionic constituents of the urine: sodium, potassium, and chloride.

METHODS

All research reported herein adheres to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Johns Hopkins University Animal Care and Use Committee. The data described herein were obtained from experiments and animals which, except for one experimental group (W-A; see below), were previously described in a companion publication (34) which reported on the effect of hydration status on urothelial water, urea, and creatinine transport. We now describe the effect of hydration status on urothelial sodium, potassium, and chloride transport in the same groups of animals using electrolyte data obtained from the same blood and urine samples as those used for previously published measurements of urea nitrogen and creatinine.

Animals, diets, and 24-h urine collection. Female Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 200–240 g, were maintained on an ad libitum intake of chow (no. 2018, Harlan Teklad Quality Lab Products) containing 18% protein, 0.23% sodium, 0.68% potassium, and 0.40% chloride and assigned to an experimental group. Rats were then placed in standard Nalgene metabolic cages and initially assigned to one of three groups: ad libitum water intake (group A), water-deprived (group D), or water-loaded (by provision of 3% sucrose in water as the sole drinking fluid; group W) for the 48 h immediately before the experimental procedure. For each rat, a 24-h urine collection was obtained immediately before the experimental procedure for purpose of confirming hydration status of the rats and for obtaining urine to be (re)instilled into the (same) rat’s bladder. All urine collections were filtered twice, first with qualitative filter paper (Whatman) to remove debris, then with a 0.2-μm-pore-size syringe filter (Corning) to remove bacteria. Preliminary experiments in which filtered urine was cultured using standard microbiological techniques confirmed that the resultant filtrates were sterile.

Animal groups. Rats were initially divided into three groups differing by state of hydration (A = ad libitum water, n = 18; D = water-deprived, n = 17; W = water-loaded, n = 24). To determine whether factors associated with the bladder or the urine (either or both of which might be altered by animal hydration status) might be the cause for differences in electrolyte transport noted in the first three groups, four additional groups of rats were subsequently studied. Thus another group of rats was water-deprived (group A-D, n = 23), and another water-loaded (group A-W, n = 19) for 48 h before the experimental procedure, but rats in these groups had their bladders instilled with urine previously (3–4 days before) obtained during ad libitum water intake. Two additional groups of rats were studied during ad libitum conditions but with urine collected during water deprivation (group D-A, n = 16) or water loading (group W-A, n = 13) 10 days earlier (animals had fully recovered from water deprivation and water loading as assessed by several 24-h urine measurements in the ad libitum state).

Experimental procedure. On the day of the experimental procedure, the 24-h urine collection was completed and filtered as described above. Then, rats were weighed, anesthetized with intraperitoneal Nembutal (60 mg/kg body wt), placed on a heated operating board, and a tracheostomy performed. A PE-50 catheter filled with sterile 154 mM sodium chloride was placed in the left carotid artery and attached to a Grass Instrument model 79E polygraph for continuous mean blood pressure recording. The left femoral vein was cannulated with a PE-50 catheter for saline infusion. Over the course of the experiment, saline was constantly infused at a rate of 0.078 ml/min to maintain blood volume and blood pressure. A midline abdominal incision was made, and both ureters were double ligated near their insertion into the bladder. Ureters were then cut proximal to the ligation, and subsequent urine was diverted exteriorly. After a 30- to 40-min equilibration time, the urinary bladder was emptied of any residual urine with gentle finger pressure on the bladder. The external urethral orifice was then catheterized with a soft tipped 22-gauge vascular catheter (Protect IV Plus Jetco Catheter, Smiths Medical) attached to a 1-ml syringe containing filtered urine (previously collected as part of the 24-h urine) warmed to body temperature. The tip of the catheter was carefully inserted into the proximal urethra at its exit from the bladder to avoid damage to the bladder urothelia. To urine the bladder of any residual urine, 0.1 or 0.15 ml of the urine previously previously collected was insilled into the bladder over 10 (0.1 ml) or 15 (0.15 ml) min. Then, the syringe was disconnected and the bladder was emptied through the catheter with gentle finger pressure on the bladder. To prevent contamination by urine solution effluent in the catheter “dead space” (0.033 ml), the urethral catheter was removed and gently replaced with a second urethral catheter connected to a syringe containing the same urine for instillation. This urine-filled syringe and catheter had been weighed to the nearest 0.001 mg. using either Mettler Toledo AB54-S or Acculab V1 analytic scales. Approximately 0.3 ml of urine warmed to body temperature was then instilled into the bladder over 30 min. The urethral catheter was then removed while the external urethral appendage was double ligated to prevent loss of bladder urine. The syringe and catheter were then weighed, and the quantity of urine instilled was taken as the weight of the urine-filled catheter and syringe minus the weight of the catheter, syringe, and residual urine remaining in the syringe after instillation. The residual urine in the syringe was then aliquotted and stored for subsequent chemical analysis as the “instilled urine” (Inst-u). The abdominal incision was then approximated and covered with warmed saline-soaked gauze. After a 60-min dwell time, the urine in the bladder was removed by puncture of an avascular site on the bladder using a 25-gauge needle attached to a 1-ml syringe. The urine so retrieved was measured gravimetrically (as syringe and urine minus empty syringe weight) and stored for subsequent chemical analysis as the “retrieved urine” (Retr-u). Whole blood was then obtained by cardiac puncture, centri-
fuged, aliquotted, and the serum was stored for subsequent chemical analysis.

Analysis of blood and urine specimens. Osmolality of urine was performed by a vapor pressure osmometer (Wescor). Serum and urine concentrations of sodium, chloride, potassium, and other analytes (including urea nitrogen and creatinine, previously reported) were made using a Hitachi 917 Analyzer (Roche Diagnostics).

Statistical analysis. Mean weight, mean arterial blood pressure, serum analytes, 24-h urine volumes, and osmolality were determined for each rat group, and group differences were analyzed using one-way ANOVA followed by the Tukey-Kramer honestly significant difference (HSD) post hoc test. For each rat, the volume (determined gravimetrically) of instilled and retrieved urine and the concentrations of instilled and retrieved urine analytes were determined, and the total number of milliequivalents were transported into, or out of, the bladder urine obtained by multiplying the (instilled or retrieved) urine volume by the (instilled or retrieved) concentrations of each analyte. Between-group differences between instilled and retrieved urine volumes, urine analyte concentrations, and total volume and analyte transport (in milliliters or milliequivalents) were also calculated using one-way ANOVA and the Tukey-Kramer HSD post hoc tests. Within-group differences of instilled and retrieved urine volumes were previously reported (34) for all groups except for group W-A. For group W-A, mean (±SE) Inst-u volume was 0.302 ± 0.000 ml; retrieved volume was 0.307 ± 0.001 ml; Retr-u − Inst-u volume was 0.005 ± 0.000 ml, representing a 1.6 ± 0.1% increase over Inst-u in the retrieved volume. These values are almost identical to those previously described in the other hydration groups (between which there were no significant differences in Inst-u, Retr-u, or change in urine volumes) and are consistent with an average 1.6% dead space artifact in the volume of retrieved urine compared with the volume of Inst-u as previously discussed (34). Thus, there was no significant change in urine volume after 1-h bladder dwell in the group W-A rats or in the rats of any other hydration group.

Changes in urine sodium concentration after bladder dwell. Mean (±SE) serum sodium concentration for groups A, W, and D were 139.6 ± 1.2, 143.4 ± 1.3, and 143.5 ± 1.1 meq/l, respectively [P = not significant (NS) for all comparisons]. Urine sodium concentrations in Inst-u and Retr-u for each individual rat (solid lines) and for the group mean (dotted line) in the three hydration groups are shown in Fig. 1. After 1-h bladder dwell, urine sodium rose in 20 of 23 water-loaded rats, fell in 15 of 17 water-deprived rats, and rose in 9 and fell in 9 of 18 rats receiving ad libitum water. Group mean urine sodium concentration (meq/l) in Inst-u and Retr-u, and differences between Inst-u and Retr-u expressed both as a numerical urine sodium concentration difference (Retr-u − Inst-u) and as a percentage difference are shown in Table 1, panel A, and numerical and percentage concentration differences are illustrated in Fig. 2, panel A. Because Inst-u and Retr-u volumes in individual rats and for groups were essentially equal, the quantity of electrolytes (volume × concentration of sodium,
Table 1. Concentration of electrolytes in instilled and retrieved urine and differences in concentration in urine dwelling for 1-h bladders of groups of rats differing in hydration status and instilled urine

<table>
<thead>
<tr>
<th>Rat hydration status</th>
<th>Panel A</th>
<th>Panel B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instilled urine, meq/l</td>
<td>Water-loaded</td>
<td>Ad libitum water</td>
</tr>
<tr>
<td>Instilled, meq/l</td>
<td>24.2 ± 1.9a</td>
<td>217.6 ± 11.0a</td>
</tr>
<tr>
<td>Retrieved urine, meq/l</td>
<td>29.1 ± 1.7a</td>
<td>124.3 ± 10.5a</td>
</tr>
<tr>
<td>Concentration difference (retrieved − instilled), meq/l</td>
<td>+4.9 ± 1.0a</td>
<td>−3.3 ± 2.8</td>
</tr>
<tr>
<td>Instilled, percentage of instilled</td>
<td>+30.7 ± 10.1ae</td>
<td>−2.1 ± 1.8</td>
</tr>
</tbody>
</table>

Concentration of sodium

| Instilled urine, meq/l | Water-loaded | Ad libitum water | Water-deprived | Water-deprived | Water-loaded | Ad libitum water | Water-deprived | Water-loaded |
| Instilled, meq/l | 26.9 ± 3.3a | 212.1 ± 16.0a | 464.8 ± 21.8a | 233.5 ± 16.8 | 214.0 ± 19.1 | 458.8 ± 17.9 | 27.5 ± 2.4 |
| Retrieved urine, meq/l | 29.5 ± 3.1a | 178.4 ± 12.0a | 305.5 ± 21.5a | 186.2 ± 14.5 | 185.2 ± 17.7 | 389.6 ± 14.3 | 32.1 ± 3.7 |
| Difference in quantity (retrieved − instilled), meq/l | +2.6 ± 1.0bP | −33.6 ± 6.6a | −159.4 ± 15.3ae | −47.2 ± 8.6a | −28.9 ± 2.7a | −69.1 ± 9.4a | +4.6 ± 2.1bP |
| Difference in quantity (retrieved − instilled), percentage of instilled | +16.5 ± 4.2a | −14.3 ± 2.0 | −34.3 ± 3.3a | −19.1 ± 2.0 | −14.2 ± 1.1 | −14.7 ± 1.9a | +15.6 ± 6.1 |

Concentration of chloride

| Instilled urine, meq/l | Water-loaded | Ad libitum water | Water-deprived | Water-deprived | Water-loaded | Ad libitum water | Water-deprived | Water-loaded |
| Instilled, meq/l | 29.8 ± 3.4a | 228.5 ± 17.7a | 376.2 ± 20.1a | 221.7 ± 17.0 | 217.5 ± 21.3 | 422.7 ± 20.6 | 31.3 ± 3.7 |
| Retrieved urine, meq/l | 35.8 ± 3.3a | 200.1 ± 15.1 | 242.5 ± 14.6a | 176.3 ± 14.2 | 185.2 ± 17.6 | 359.1 ± 18.2 | 36.2 ± 3.9 |
| Difference in quantity (retrieved − instilled), meq/l | +6.0 ± 0.9a | −28.4 ± 6.3a | −133.7 ± 21.4ae | −44.8 ± 9.3a | −32.5 ± 7.4a | −63.6 ± 8.1a | +4.9 ± 1.1a |
| Difference in quantity (retrieved − instilled), percentage of instilled | +26.8 ± 5.3a | −12.0 ± 2.0 | −33.8 ± 4.2a | −19.5 ± 3.4 | −13.3 ± 2.9 | −14.9 ± 1.9a | +19.7 ± 4.8 |

Values are means ± SE. *P < 0.0001 retrieved vs. instilled. †P < 0.02 retrieved vs. instilled. For panel A, *P < 0.0001 vs. other groups. †P < 0.02 vs. group W. ‡P < 0.0001 vs. group D. ‡P < 0.002 vs. group A. *P = 0.04 vs. group A. For panel B, *P = 0.05 vs. group D. ‡P < 0.0001 vs. group D. ‡P < 0.002 vs. group D. *P = 0.02 vs. group D.

potassium, and chloride, in meq) in Inst-u and Retr-u and bladder dwell changes in quantity and percentage of electrolytes for individual rats and for all groups closely followed the same pattern as for electrolyte concentrations, and therefore only electrolyte concentrations will be presented herein. As expected, urine sodium concentration in Inst-u was significantly different between groups, being lowest in the urine of water-loaded animals and highest in urine of water-deprived animals (Table 1, panel A). The change in urine sodium concentration also differed between hydration groups. While there was no significant change in urine sodium concentrations after 1-h dwell in the ad libitum rats, there was a small but significant numerical increase (4.9 meq/l) in urine sodium concentration during bladder dwell in water-loaded rats (a 30.7% increase over Inst-u sodium concentration representing sodium secretion from the urethra layer into urine), and a larger, significant numerical decrease (53.2 meq/l) in urinary sodium concentration during bladder dwell in water-deprived rats (an 18.6% decrease from Inst-u sodium concentration representing sodium reabsorption from urine into bladder tissues) (Table 1, panel A; Fig. 2A).

Changes in urine sodium concentration after 1-h bladder dwell were determined, at least in part, by the urine sodium concentration (or an associated urine factor) in the Inst-u. Figure 3A shows a significant association of the concentration of sodium in Inst-u with change in urine sodium concentration in the Retr-u after the 1-h bladder dwell. The solid black line in the figure represents the overall linear regression model of the combined A, D, and W groups. The regression equation was the following: slope of the sodium concentration change (meq/l) = −0.2883 × Inst-u sodium concentration [95% confidence intervals (CI) for the slope were −0.3518, −0.2249, P < 0.0001], suggesting that at least part of the change in urine sodium concentration after the 1-h bladder dwell was a function of the sodium concentration in the Inst-u.

To determine whether sodium secretion and/or reabsorption are solely dependent on urine factor(s) or bladder factors, or both, we studied four additional groups of animals (groups A-D, A-W, D-A, and W-A) previously described in METHODS. Inst-u and Retr-u volumes for these additional groups were not significantly different from the three original groups, nor were the net changes in urine volume, whether expressed in milliliters or as a percentage of instilled volume.

Changes in urine sodium concentration in the additional rat groups. Urine sodium concentration in Inst-u and Retr-u and differences in concentration between Inst-u and Retr-u in the four additional rat groups are shown in Table 1, panel B, and the differences are illustrated in Fig. 2B. Concentration of Inst-u sodium was not different between groups instilled with urine collected under ad libitum conditions (groups A-D, A-W, and A), or between groups instilled with urine collected under water-loaded conditions (groups W-A and W) but was higher (P = 0.05) in group D-A than in D. In the additional groups, there was either a significant reduction (groups A-D, A-W, and D-A) or increase (group W-A) in urine sodium concentration in the Retr-u compared with that in Inst-u. There was no difference in change of concentration (whether expressed numerically in meq/l or as a percentage) between groups whose bladders were infused with similarly collected urine, regardless of the hydration state. Thus there was no difference in change of concentration among groups A-D, A-W, and A, between groups D-A and D, or between groups W-A and A (all P = NS; Table 1, panels A and B, and Fig. 2, A and B).
The similarities in concentration changes between groups differing in hydration status but instilled with urine obtained during ad libitum water intake (groups A, A-D, and A-W) and during water loading (groups W and W-A) suggested that urine sodium concentration, and not hydration status, largely determined transurothelial sodium transport at “lower” to “mid” levels of instilled urinary sodium. However, despite having a significantly higher concentration of urinary sodium in Inst-u, group D-A rats tended (P = NS) to have a lesser fall of urinary sodium concentration during bladder dwell than the water-deprived rats (group D), suggesting that during water deprivation the bladder might allow greater sodium reabsorption than the bladder during ad libitum water conditions. To test this possibility, we performed additional regression analysis by plotting Inst-u sodium concentration and change in sodium concentration and compared all rats studied in the ad libitum water condition (groups A and D-A) and all rats studied in the water-deprived condition (groups D and A-D). The slope of the regression line (data not illustrated) for the water-deprived groups was $-0.4184 \times$ Inst-u sodium concentration (95% CIs = $-0.5158$, $-0.3211$, $P < 0.0001$), more than twice that of the ad libitum rats (slope = $-0.1730$; CIs = $-0.2296$, $-0.1163$, $P < 0.0001$), suggesting that water deprivation increased net bladder sodium reabsorption via a bladder tissue mechanism(s) independent of urine sodium concentration.

Changes in urine potassium concentration after bladder dwell. Mean (±SE) serum potassium concentration for groups A, W, and D were 6.1 ± 0.2, 5.8 ± 0.2, and 5.6 ± 0.2 meq/L, respectively (P = NS for all comparisons). Urine potassium concentrations in Inst-u and Retr-u for each individual rat (solid line) and for the group means (dotted lines) in the three hydration groups are shown in Fig. 4. After 1-h bladder dwell, urine potassium concentration rose in 15 of 23 water-loaded rats and fell in all 17 water-deprived rats and in 17 of 18 rats receiving ad libitum water.

Fig. 2. Change in urine sodium concentrations after 1-h bladder dwell expressed as meq/L (open bars) and as a percentage (solid bars) in water-loaded (group W), ad libitum water (group A), and water-deprived (group D) groups of rats (panel A) and in 4 additional groups of rats (panel B). Group A-D, water-deprived rats whose bladders were instilled with urine obtained during ad libitum conditions; group A-W, water-loaded rats whose bladders were instilled with urine obtained during ad libitum conditions; group D-A, ad libitum water rats whose bladders were instilled with urine collected under water-deprived conditions; group W-A, ad libitum water rats whose bladders were instilled with urines collected under water-loaded conditions. Values are means ± SE. A: *P < 0.0001 vs. groups W and A. +P < 0.0002 vs. group A, <0.0001 vs. group D. B: P = not significant (NS), group D-A vs. group D, group W-A vs. group W, and groups A-D and A-W vs. group A.

Fig. 3. Plots of instilled urine concentrations (meq/L) of sodium (A), potassium (B) and chloride (C) on the x-axis vs. change in urine concentration of each analyte on the y-axis for all animals in the original 3 hydration groups (W, A, and D). Each point represents a single animal. For each analyte, there is a significant relationship between instilled urine concentration and change in urine concentration; the slope equations are given the text.
Group mean urine potassium concentration (meq/l) in Inst-u and Retr-u as well as differences between Inst-u and Retr-u are shown in Table 1, panel A, and numerical and percentage concentration differences are illustrated in Fig. 5A. Urine potassium concentration in Inst-u was significantly different between groups, being lowest in urine of water-loaded animals and highest in water-deprived animals (Table 1, panel A). During 1-h bladder dwell, mean urine potassium concentration rose significantly (2.6 meq/l or 16.5%, representing secretion from epithelia into urine) in water-deprived rats but fell in ad libitum rats (−33.6 meq/l, −14.8%) and water-deprived rats (−159.4 meq/l, −34.6%), representing potassium reabsorption in the latter two groups. Both the numerical and percentage changes in potassium concentration were significantly different between groups (Table 1, panel A; Fig. 5A).

To evaluate whether the changes in urine potassium concentration after 1-h bladder dwell were determined, at least in part, by the urine potassium concentration (or an associated urine

![Urine potassium concentrations](image)
factor) in the Intr-u, we performed regression analysis by plotting instilled potassium concentration and change in potassium concentration during bladder dwell using all animals in the three hydration groups. Figure 3B shows a significant association between the potassium concentration in Inst-u and the change in potassium concentration in the Retr-u after 1-h bladder dwell. The solid black line in the figure represents the overall linear regression model of all groups combined. The regression equation was the following: slope of the potassium concentration change (meq/l) = \(-0.3599 \times \) Inst-u potassium concentration (95% CIs for the slope \(-0.4113, -0.3087, P < 0.0001\)), suggesting that the change in urine potassium concentration after the 1-h bladder dwell was, at least in part, a function of the potassium concentration in the instilled urine.

To determine whether potassium secretion was solely dependent on a urine factor(s), or bladder factors, or both, we studied four additional animal groups (previously described).

**Changes in urine potassium concentration in the additional rat groups.** Urine potassium concentration in Inst-u and Retr-u and differences in concentration between Inst-u and Retr-u in the additional four groups are shown in Table 1, panel B and Fig. 5B. Concentration of Inst-u potassium was similar (all comparisons \(P = \text{NS}\)) in groups instilled with urine collected under ad libitum conditions (groups A-D, A-W, and A), under water-loaded conditions (groups W and W-A), and under water-deprived conditions (groups D and D-A). In the additional groups, there was a significant reduction in urine potassium concentration in the Retr-u compared with that in the Inst-u in groups A-D, A-W, and D-A and an increase in group W-A (Table 1, panel B). There were no differences (all \(P = \text{NS}\)) in the change in potassium concentration (whether expressed numerically as meq/l or as a percentage of instilled potassium concentration) between groups instilled with urine collected during water-loaded conditions (groups W and W-A) or among groups instilled with urine collected during ad libitum water conditions (groups A, A-W, and A-D), suggesting that Inst-u potassium concentration played a predominant role in urothelial transport at low to mid-potassium concentrations. However, there was a large and significant \((P < 0.0001)\) difference in the fall of urinary potassium concentration (potassium reabsorption) between the water-deprived (D; \(-159.4\) meq/l, \(-34.6\%\)) and the D-A rat groups (\(-69.1\) meq/l, \(-14.7\%\)), suggesting that animal hydration status (independent of hydration effect on urine potassium concentrations) might in part determine potassium reabsorption, at least when Inst-u contains a high potassium concentration. To further test this possibility, we performed additional regression analysis by plotting Inst-u potassium concentration and change in potassium concentration and compared all rats studied during ad libitum water conditions (groups A and D-A) and all rats studied during water deprivation (groups D and D-A). The slope of the regression line for change in urinary potassium concentration for rats studied during water deprivation was \(-0.4072 \times \) Inst-u potassium concentration (CIs \([-0.5190, -0.2953, P < 0.0001]\)), more than twice that of rats receiving ad libitum water (slope \(-0.1826 \times \) Inst-u potassium concentration; CIs \([-0.2489, -0.1164, P < 0.0001]\)), further suggesting that water deprivation increased net bladder potassium reabsorption via a bladder mechanism independent of urine potassium concentration.

**Changes in urine chloride concentration after bladder dwell.** Mean (\(\pm SE\)) serum chloride concentrations for groups A, W, and D were 105.5 \(\pm 1.6\), 108.3 \(\pm 1.5\), and 106.9 \(\pm 1.2\) meq/l, respectively \((P = \text{NS}, \text{all comparisons})\). Urine chloride concentration in Inst-u and Retr-u for each individual rat (solid lines) and for the group mean (dotted lines) in the three hydration groups are shown in Fig. 6. After 1-h bladder dwell, urine chloride concentration rose in 23 of 23 water-loaded rats and fell in 17 of 18 rats receiving ad libitum water, and in 16 of 17 water-deprived rats. Group mean urine chloride concentrations (meq/l) in Inst-u and Ret-u, as well as differences between Inst-u and Ret-u expressed both in meq/l and as a percentage of Inst-u are shown in Table 1, panel A and

![Fig. 6. Urine chloride concentrations (meq/l) in instilled and retrieved urine for each rat in the 3 hydration groups. The solid lines connect the instilled and retrieved chloride concentration (●) for individual rats; ▲ and dotted lines represent mean values for each group. *\(P < 0.0001\) instilled vs. retrieved urine chloride concentration.](image-url)
illustrated in Fig. 7A. As was the case for other analytes, Inst-u urine chloride was lowest in urine of water-loaded rats, and highest in urine of water-deprived rats. After 1-h bladder dwell, mean urine chloride rose significantly in water-loaded rats (6.0 ± 0.9 meq/l, a 26.5 ± 5.3% increase above Inst-u, representing secretion into urine) and fell both in rats receiving ad libitum water (−28.4 ± 6.3 meq/l, −12.0 ± 2%) and in water-deprived rats (−133.7 ± 2.14 meq/l, −33.8 ± 4.2%), representing reabsorption from bladder urine. As was the case for sodium and potassium, regression analysis (Fig. 3C) showed a significant association of the change in chloride concentration (after 1-h bladder dwell) with the Inst-u chloride concentration. The solid black line in the figure represents the overall linear regression model for all groups combined. The regression equation was the following: slope of the chloride concentration regression model for all groups combined. The regression equation was the following: slope of the chloride concentration regression model for all groups combined.

The similarities in concentration changes between groups differing in hydration status but instilled with urine obtained during ad libitum water intake (groups A-D, A-W, and A) and during water loading (groups W and W-A) suggested that urine chloride concentration, and not hydration status, largely determined transtuberothelial chloride transport at lower to mid-levels of urinary chloride. However, the fact that the water-deprived group (group D) had a greater fall in urinary chloride concentration than group D-A suggested that the water-deprived state per se, independent of urinary chloride concentration, might result in increased chloride reabsorption compared with the ad libitum water condition. To further test this possibility, we performed additional regression analysis by plotting Inst-u chloride concentration (95% CIs for the slope = −0.4736, −0.3237, \(P = 0.0001\)), suggesting that the change in urine chloride concentration after the 1-h bladder dwell was a function of the chloride concentration in Inst-u.

To determine whether chloride secretion and/or reabsorption are solely dependent on a urine factor(s), or bladder factors, or both, we studied four additional groups of animals, as previously discussed.

Changes in urine chloride concentration in the additional rat groups. Urine chloride concentration in Inst-u and Retr-u and differences in concentration between Inst-u and Retr-u in the four additional rat groups are shown in Table 1, panel B, and Fig. 7B. Concentration of Inst-u chloride was not different in groups instilled with urine collected under ad libitum conditions (groups A-D, A-W, and A), water-deprived conditions (groups D-A and D), and water-loaded conditions (groups W-A and W). Retr-u chloride concentration in group W-A rose 4.9 ± 1.1 meq/l or 19.6%, similar to the water-loaded group. Retr-u chloride in groups A-W and A-D fell −32.5 and −44.8 meq/l (−13.3 and −19.5%, respectively), similar to the fall of −28.4 meq/l (−12.0%) in the ad libitum water group (all group comparisons \(P = NS\)). However, the absolute and percent reductions in urine chloride concentration were significantly less in group D-A (−63.6 ± 8.1 meq/l or −14.9 ± 1.9%) than in the water-deprived (−133.7 ± 21.4 meq/l, −33.8%) group (Table 1, Fig. 7).

The similarities in concentration changes between groups differing in hydration status but instilled with urine obtained during ad libitum water intake (groups A-D, A-W, and A) and during water loading (groups W and W-A) suggested that urine chloride concentration, and not hydration status, largely determined transtuberothelial chloride transport at lower to mid-levels of urinary chloride. However, the fact that the water-deprived group (group D) had a greater fall in urinary chloride concentration than group D-A suggested that the water-deprived state per se, independent of urinary chloride concentration, might result in increased chloride reabsorption compared with the ad libitum water condition. To further test this possibility, we performed additional regression analysis by plotting Inst-u

Fig. 7. Change in urine chloride concentrations after 1-h bladder dwell expressed as meq/l (open bars) and as a percentage (solid bars) in group W, group A, and group D rats (panel A) and in 4 additional groups of rats (panel B). All groups are defined as in Fig. 2. Values are means ± SE.

A: \(*P < 0.0001 vs. other groups, \*P = 0.003 vs. group A,

B: \(\Psi < 0.0001 vs. group D, \**P = 0.02 vs. group D.\)
chloride concentration and change in chloride concentration and compared all rats studied in the ad libitum water condition (groups A and D-A) and all rats studied in the water-deprived condition (groups D and A-D; data not illustrated). The slope of the regression line for the change in urine chloride concentration for the water-deprived groups was $-0.5446 \times \text{Inst-u chloride concentration}$. The CI for $t = -0.0939$, $P < 0.0001$, more than twice that of the ad libitum rats (slope = $-0.1802$, CI = $-0.2581$, $P < 0.0001$), further suggesting that water deprivation increased net chloride reabsorption via a bladder (urothelial) mechanism independently of urine chloride concentration, as was also the case for reabsorption of sodium and potassium.

**DISCUSSION**

Herein, using a new rat model to study in vivo mammalian solute transport between urine and bladder tissues under physiological conditions, we report both net urothelial secretion and net urothelial reabsorption of urinary sodium, potassium, and chloride. Although the mammalian lower urinary tract is generally considered an impermeable storage and transit vehicle (12, 13, 17, 28), support for our findings comes from a number of prior reports of net ion transport across mammalian urothelia (8, 11, 14–16, 19, 23, 25, 26, 31, 42, 43) and from reports describing the presence of a number of ion transporters in lower urinary tract epithelial cells (see the beginning of this paper; reviewed in Ref. 21). Since many of these transporters play a role in net vectorial ion transport across renal epithelial cells, it seems plausible that a similar role is being played by the same transporters in urothelia.

We further report, for the first time, that hydration status plays an important role in determining the direction and magnitude of net urothelial transport for sodium, potassium, and chloride. Thus during ad libitum water intake the concentration of urinary potassium and chloride fell 14.8 and 12%, respectively, during 1-h bladder dwell, representing reabsorption from urine across the urothelial cell layer lining the bladder into deeper bladder tissues. There was no significant transport of urinary sodium (a fall of 2.1%, $P = \text{NS}$). During water deprivation, urine concentration of ionic species in bladder urine fell by 18.6, 34.6, and 33.8%, respectively, for sodium, potassium, and chloride, reflecting dramatically greater net ion reabsorption than was the case during ad libitum water intake. In contrast, during whole animal water-loading conditions the urinary concentration of all three ions rose a small but significant amount, 16.5% for potassium, 26.8% for chloride, and 30.7% for sodium, representing secretion of ions from urothelia into urine. Thus the direction and magnitude of net transport (as a percentage of instilled ion) during the various hydration states for each ionic species were similar to what we previously reported (34) for urinary urea nitrogen and creatinine in the same animals.

Finally, we found that, as was the case for urea and creatinine (34), the magnitude of the concentration changes during bladder dwell for each ion is a function of both the urinary concentration of the instilled ion and the animal’s hydration state. Thus for each of the three ionic species, maximum electrolyte reabsorption was achieved only in the water-deprived rats whose bladders were instilled with urine obtained during water deprivation. These novel findings demonstrate that at least two factors linked to an animal’s hydration status independently mediate the effect(s) of that state on urothelial ion transport: a urine factor(s), likely the urinary ionic concentration (but possibly a urinary factor which cosegregates with ion concentrations), and a factor associated with the bladder urothelia itself, presumably an alteration of one or more of the three reported permeability ‘barriers’ residing in urothelia [the apical membrane of the luminal “umbrella” cells, the tight junctions between those cells, and/or the glycosaminoglycans (GAGS) layer overlying those cells] (24, 29, 34). Additional transport studies in groups of rats treated identically to those described herein but utilizing “artificial urine” solutions containing the same ion and solute concentrations as in native urine might help define urine factors regulating urothelial ion, solute, and even water transport.

The mechanism(s) whereby net transurothelial transport of sodium, chloride, and potassium occurs are not clear. Simple passive diffusion across urothelia may well play some role given that for most hydration groups the direction and magnitude of transport for each ionic species (as well as for urea and creatinine, previously described) (34) are similar and down the apparent urine-plasma concentration gradient. The lack of significant net transport of sodium in the ad libitum water group may reflect the similar sodium concentrations in, and lack of large concentration gradient between, instilled urine and plasma. Conversely, simple diffusion down a plasma-urine gradient could not account for the observed secretion of potassium during water-loading conditions, although it could occur down a presumed intracellular-urine potassium concentration gradient. Furthermore, the speed and magnitude of solute transport in our studies as well as in those of Levinsky and Walser (23, 42) seem higher than what might be attributed to simple passive diffusion and suggest facilitated and/or active transport. In this regard, it is notable that a number of facilitative and active channels and transporters have been described in mammalian urothelial cells. The best described urothelial ion transporter is the amiloride-sensitive epithelial sodium channel (ENaC), which has been extensively studied in urothelia (6, 22, 24–26, 33, 43). ENaC is located on the apical membrane (33) and may have several important physiological roles, including apical membrane sodium transport, modulation of urothelial cell ATP release (9), and modulation of stretch-induced exocytosis of the umbrella cell apical membrane (reviewed in Ref. 21). At least one chloride transport system is known to be present in urothelia: a stretch-mediated, electroneutral chloride and potassium transport in part mediated by an apically expressed nonselective cation channel (NSCC) (43). In contrast to sodium and chloride transporters, multiple potassium transporters have been described in urothelial cells including: the renal outer medullary K+ channel (ROMK, K$_{ir}$ 1.1) located on the apical membrane (37); a heparin-binding, EGF-modulated inwardly rectifying channel (KCNN1–4), two two-pore (BK) channel, a small or intermediate-conductance K$^+$ channel (Kir 2.1, 2.4); and the large-conductance (Maxi-K) channel (40). More recently, Yu and coworkers (46) identified the (message) presence of multiple urothelial K$^+$ channels, including the large-conductance K$^+$ (BK) channel, a small or intermediate-conductance K$^+$ channel (KCNN1–4), two two-pore K$^+$ channels (TREK-1, TRAAK), and two inwardly rectifying ATP-modulated K$^+$ channels (K$_{atp}$ 6.1, K$_{atp}$ 6.2). The function(s) of these transporters in the urothelium remain(s) unknown, but in renal epithelia cells some are known to mediate vectorial...
membrane transport and these may well function similarly in urothelial cells. If so, their most likely localization might be expected on the apical membrane of the umbrella cells lining the urinary tract mucosa as is the case for ROMK (37) since this membrane otherwise represents one of the three urothelial barriers to solute and water transport (21, 24, 29). Future studies in this same rat model using systemic and/or mucosal application of channel blockers and transport inhibitors (e.g., amiloride for ENaC) might be useful in defining the role of such channels and transporters in urothelial transport. It is possible that hydration status might have regulated urothelial ion transport by directly affecting specific urothelial channels and transporters, and thereby specific solute net transport. However, given that water deprivation resulted in similar increases in percentage of reabsorption and water loading in similar increased secretion, of most of the urinary solutes studied to date it seems more likely that hydration status affected either the umbrella cell tight junctions, the mucosal surface GAGS layer, or some integral component of the apical membrane, as we previously discussed regarding the effect of hydration status on urea and creatinine transport (34). Alteration of one or more of these permeability barriers might thus account for the simultaneous and similar increase in permeability for multiple solutes in our studies. A potential mechanism whereby one or more permeability barriers might be altered could relate to the profound changes in urothelial cell size and shape associated with cyclic bladder filling and emptying. Filling is associated with stretching and flattening of urothelial cells and an increase in umbrella cell apical membrane surface area due to exocytic incorporation of cytoplasmic discoidal/fusiform vesicles (DFV) into the membrane. (Simultaneously there is also increased endocytosis of apical membrane into vesicles.) Since DFVs contain ENaC and likely other transporter proteins, DFV cycling may promote changes in ion and solute transport, and indeed functional studies in Ussing chambers have shown that stretching of the umbrella cells resulted in increased sodium reabsorption (via ENaC) and secretion of potassium and chloride (43). Furthermore, as we have previously speculated, DFV cycling may promote net ion/solute transport directly by incorporating urinary constituents into endocytic vesicles and cytoplasmic ions and solutes into exocytic vesicles (35). Little is known about the possible effect(s) of bladder stretch on the GAGs layer lining the bladder lumen or the permeability characteristics of the umbrella cell tight junctions. Differences in stretch did not appear to mediate differences in ion transport between our water-loaded and ad libitum rat groups, at least, since changes in transported ions were similar in groups receiving similarly concentrated urine regardless of rat hydration status.

Given that the major role of the mammalian lower urinary tract is to provide for transport and storage of urine made by the kidney, it is not clear what biological advantage is subserved by urothelial sodium, potassium, and chloride transport, although roles in urothelial autocrine and/or paracrine signaling or in local urothelial and/or systemic solute and water homeostasis are possible. Recently, the urothelium has been shown to be directly involved in what has been termed the “uroepithelial-associated sensory web” (1). This web includes the urothelium as well as subepithelial tissues, including afferent and efferent nerves, smooth muscle cells, and myofibroblasts, among which there is considerable molecular communi-

cation subserving bladder functions including muscular contraction and relaxation, cell membrane turnover, and important sensory functions (1–3, 9, 10, 20, 22). Solute transport across urothelia, in particular sodium transport (likely related to the mechanosensitive ENaC sodium channel) and potassium transport may play a direct or indirect signaling role in this setting. It may therefore be no coincidence that a potassium sensitivity test has been used to characterize loss of the urothelial permeability barrier in lower urinary tract diseases including interstitial cystitis, urethritis, prostatitis, and other diseases associated with symptoms of urgency, frequency, and urinary tract pain (29). This test utilizes a brief instillation of a potassium solution into the bladder, resulting in bladder irritation and urgency in afflicted patients but not in normal patients. Interestingly, instillation of sodium instead of potassium causes no symptoms, and instillation of heparin, which restores mucosal GAGs, ameliorates the symptoms, suggesting both the specificity of potassium as a possible ionic mediator of bladder pain sensation and the importance of GAGs as a barrier to urothelial solute transport (29, 30).

Since we observed no net transport of water despite differing water conditions, our studies do not support a direct effect on total body water homeostasis. However, they do suggest a potential and unexpected role in whole body homeostasis of the major urinary solutes, resulting in a blunting of the loss of urinary solutes during ad libitum and water-deprived conditions and by slightly increasing solute loss during water-loading conditions. To further define these relationships, future studies utilizing a range of dietary intakes of sodium, potassium, and chloride would likely be fruitful.

In summary, using an in situ rat bladder model designed to study urothelial solute and water transport under physiological conditions, we describe net urothelial secretion of sodium, potassium, and chloride during water-loading conditions and net urothelial reabsorption during ad libitum water intake and, especially, during water deprivation. Two factors related to hydration status, the urinary ionic concentration and a urothelial (bladder) factor, independently affect the direction and magnitude of transport. Transport of these ions may play roles in signaling between urothelial cells and the subepithelial tissues responsible for muscular contraction/relaxation and bladder sensory output, in local tissue and whole body solute homeostasis, and in diverse lower urinary tract disease states including prostatitis, urethritis, cystitis, bladder outlet obstruction, and aging.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.A.S. and J.D. provided conception and design of research; D.A.S. and J.D. performed experiments; D.A.S., J.D., and K.J.S. analyzed data; D.A.S., J.D., and K.J.S. interpreted results of experiments; D.A.S. and J.D. prepared figures; D.A.S. and K.J.S. drafted manuscript;
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