An Independent Effect of Osmolality on Urea Transport in Rat Terminal Inner Medullary Collecting Ducts

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Abstract

We have shown that urea transport across the terminal inner medullary collecting duct (terminal IMCD) is mediated by a vasopressin-stimulated, facilitated diffusion process exhibiting properties consistent with a transporter. To investigate whether hypertonic NaCl, as exists in vivo in the inner medulla, affects urea permeability, we studied isolated perfused rat terminal IMCD segments. Perfusate and bath osmolality were varied symmetrically by adding or removing NaCl or mannitol. Urea permeability rose progressively as osmolality was increased with NaCl or mannitol from 290 to 690 mOsm/kg H2O in the absence of vasopressin; there was no further increase at 890 mOsm/kg H2O. In the presence of 10^-8 M arginine vasopressin, urea permeability increased when NaCl was added to raise osmolality from 290 to 490 mOsm/kg H2O but there was no further increase at 690 mOsm/kg H2O. When 1 mM 8-bromo cyclic AMP was added to the bath, raising NaCl still increased urea permeability. These results suggest that urea transport across the rat terminal IMCD is regulated both by vasopressin and by osmolality at values present in the renal inner medulla. Osmolality seems to activate urea transport across the rat terminal IMCD by mechanisms distinct from those of vasopressin or cyclic AMP. (*J. Clin. Invest. 1991. 88:137-142.) Key words: NaCl • vasopressin • cyclic AMP • concentrating mechanism • hypertonicity

Introduction

Production of a concentrated urine requires that urea be delivered to the deepest portion of the renal inner medulla (1). This is possible because urea reabsorption is sharply limited until the terminal inner medullary collecting duct (terminal IMCD), located in the deepest two-thirds of the inner medulla (2, 3). We found that urea flux across the terminal IMCD involves a facilitated transport mechanism which is stimulated by vasopressin but does not require energy (3). To date, no transport protein has been isolated. Evidence for a specific urea transporter in the terminal IMCD includes: (a) urea transport occurs too rapidly for simple paracellular transport or lipid-phase diffusion (3), (b) urea transport is inhibited by phloretin and urea analogs (4), (c) solvent drag does not explain urea transport (5, 6), and (d) there is saturation of thiourea transport (7).

During antidiuresis, the process of countercurrent multiplication generates steep axial gradients of NaCl and urea within the inner medulla. The physiology of concentrating the urine requires rapid transport of urea into the interstitium (8, 9). Vasopressin is important because it activates water reabsorption, thereby raising the concentration of urea in the tubule fluid reaching the terminal IMCD. If interstitial osmolality activated urea transport, it would augment urea delivery within the terminal two-thirds of the IMCD closest to the papillary tip. In the present studies, we explored the role of hypertonicity in regulating urea transport across the terminal IMCD to address three questions: (a) does urea permeability increase in response to hypertonicity or to increasing NaCl alone; (b) is vasopressin necessary for a change in urea permeability occurring with changes in osmolality; and (c) does a response to NaCl and vasopressin involve the same pathway?

Methods

Experimental animals

Pathogen-free male Sprague-Dawley rats weighing 75-120 g (Harlan Sprague Dawley Inc., Indianapolis, IN) were kept in filter-top cages with autoclaved bedding and fed autoclaved rat chow. The animals were maintained on a rat chow (NIH-31M; Ziegler Brothers Inc., Gardner, PA) which contained 56 mEq/kg Na+ and 259 mEq/kg K+ and were given food and water ad libitum. 20 min before the experiment, the animals were injected with furosemide (1 mg/100 g BW IP) and terminal IMCD segments were dissected using methods described previously (2, 3).

Coronal slices of kidneys were transferred to a dissection dish containing chilled dissection solution (17°C) which contained (in millimolar): NaCl, 118; NaHCO3, 25; CaCl2, 2; KHPO4, 2.5; MgSO4, 1.2; glucose, 5.5; and creatinine, 4. Terminal IMCDs were dissected from a region 40-70% of the distance between the inner-outer medullary junction and the papillary tip and were perfused as described previously (10). After the tubule had been mounted on the pipettes, it was warmed to 37°C and allowed to equilibrate for 40 min before starting any collections (3). The same solution was used in the perfusate and bath except that 5 mM urea was added to the bath solution and 5 mM raffinose to the perfusion solution. This creates a 5 mM bath-to-lumen urea gradient without an osmotic gradient (2). To avoid dissipation of the transepithelial urea gradient, perfusion rate was varied to maintain the collected urea concentration under 2.5 mM. Urea permeability did not vary with perfusion rate (Figs. 1 and 2) and was stable for up to 1 h at each osmolality (Fig. 2). The solution was gassed continuously with 95% O2 and 5% CO2 before and during the dissection (2, 3). Osmolality

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1. Abbreviation used in this paper: IMCD, inner medullary collecting duct.
Experimental protocols

**Effect of varying osmolality in the absence of vasopressin.** After the 40-min equilibration period, three to four collections (~50 nl each) were obtained while perfusate and bath osmolality was 290 mOsm/kg H$_2$O. Next, both perfusate and bath osmolality were increased to 490 mOsm/kg H$_2$O, 690 mOsm/kg H$_2$O, and 890 mOsm/kg H$_2$O by adding either NaCl or mannitol; three to four additional collections were made at each osmolality. As a control for the effects of time and increasing osmolality, half of the tubules were studied at 890 mOsm/kg H$_2$O in the first period, followed by reducing the osmolality progressively to 690 mOsm/kg H$_2$O, 490 mOsm/kg H$_2$O, and 290 mOsm/kg H$_2$O in subsequent periods. These tubules were dissected in isotonic medium and perfused with isotonic perfusate and bath for 15 min. Perfusate and bath osmolalities were then increased to 890 mOsm/kg H$_2$O for the remainder of the 40-min equilibration period.

**Effect of varying osmolality with vasopressin.** After the equilibration period and baseline collections when perfusate and bath osmolalities were 290 mOsm/kg H$_2$O, vasopressin (10 nM) or 8-bromo cyclic AMP (1 mM) was added to the bath and three to four additional collections were obtained. Subsequently, both perfusate and bath osmolality were increased to 490 mOsm/kg H$_2$O and 690 mOsm/kg H$_2$O by adding NaCl. There were three to four additional collections at each osmolality in the presence of vasopressin or 8-bromo cyclic AMP. Previously we found that the water flux ($J_w$) is 0 under these conditions (reference 5) and unpublished data of Sands, J. M., H. Nonoguchi, and M. A. Knepper). As before, perfusate and bath osmolalities were increased to 690 mOsm/kg H$_2$O at the same time that vasopressin or 8-bromo cyclic AMP was added to the bath and osmolalities were decreased to 490 mOsm/kg H$_2$O and 290 mOsm/kg H$_2$O and collections made in the presence of vasopressin or 8-bromo cyclic AMP in half of the tubules.

**Urea measurement.** The urea concentration in perfusate, bath, and collected fluid was measured using a continuous-flow ultramicro-fluorometric measurement (2). The method uses two enzymatic reactions: (a) conversion of urea to ammonia catalyzed by urease, and (b) reaction of the ammonia with alpha-ketoglutarate and NADH to form NAD$^+$, catalyzed by glutamate dehydrogenase (Kit #65-A; Sigma Chemical Co., St. Louis, MO). The disappearance of NADH is proportional to the urea present in the injected sample. The method is linear from 0–60 pmol urea and is capable of resolving differences of 4% or greater in urea concentration (2).

The urea transport rate is calculated as: $J_u = C_uV_p - C_bV_b$, where $C_u$ is the urea concentration in the perfusate, $C_b$ is the urea concentration in the collected fluid, $V_p$ is the perfusion rate per unit tubule length, and $V_b$ is the collection rate per unit tubule length. As $C_b = 0$ in these experiments, this equation simplifies to: $J_u = -C_uV_b$. The urea permeability is calculated from the urea transport rate ($J_u$) as: $P_u = J_u/(rID \times C_{im})$, where $C_{im}$ is the log-mean urea concentration difference along the tubule and ID is the tubule inner diameter.

**Results**

**Effect of changing osmolality with NaCl.** Urea permeability increased as osmolality was raised from 290 mOsm/kg H$_2$O to 690 mOsm/kg H$_2$O by the addition of NaCl in the absence of vasopressin (Table I). There was no further increase in urea permeability at 890 mOsm/kg H$_2$O and there was no difference in the response of tubules in which osmolality was progressively increased from 290 mOsm/kg H$_2$O to 890 mOsm/kg H$_2$O (Fig. 3, solid lines) or decreased from 890 mOsm/kg H$_2$O to 290 mOsm/kg H$_2$O (Fig. 3, dashed lines).

**Effect of changing osmolality with mannitol.** Urea permeability (in the absence of vasopressin) also increased when mannitol was used to raise osmolality from 290 mOsm/kg H$_2$O to 490 mOsm/kg H$_2$O; at 490 mOsm/kg H$_2$O a plateau value was reached (Table II). There was no difference in the response of tubules in which osmolality was progressively increased

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2. Although not published in reference 7, we measured water flux ($J_w$) in the six tubules presented in Fig. 1 of that paper, using methods described in reference 5. In the presence of vasopressin, $J_w$ was $-0.45 \pm 0.70$, not significantly different from 0 when perfusate and bath osmolalities were 680 mOsm/kg H$_2$O. These results are consistent with the absence of a transepithelial osmotic gradient (unpublished data of Sands, J. M., H. Nonoguchi, and M. A. Knepper).
from 290 mOsm/kg H₂O to 890 mOsm/kg H₂O (Fig. 4, solid lines) or progressively decreased from 890 mOsm/kg H₂O to 290 mOsm/kg H₂O (Fig. 4, dashed lines). When the effects of mannitol and NaCl were compared, urea permeability was significantly lower (by ANOVA) at 690 mOsm/kg H₂O with mannitol but not at other osmolalities tested (Tables I and II).

Effect of changing osmolality in the presence of vasopressin. Urea permeability also increased when NaCl was added (290 mOsm/kg H₂O) in the presence of 10 nM vasopressin at 290 mOsm/kg H₂O (Table III). Permeability increased further as osmolality was increased from 290 mOsm/kg H₂O to 490 mOsm/kg H₂O but not at 690 mOsm/kg H₂O (Table III). Again, there was no difference in the response of tubules when the osmolality was progressively increased from 290 mOsm/kg H₂O to 690 mOsm/kg H₂O (Fig. 5, solid lines) or decreased from 690 mOsm/kg H₂O to 290 mOsm/kg H₂O (Fig. 5, dashed lines). The increase in permeability from 290 mOsm/kg H₂O to 490 mOsm/kg H₂O in the presence of a supraphysiologic concentration of vasopressin was not different statistically (by t test) from the change induced by osmolality alone.

Effect of changing osmolality in the presence of 8-bromo cyclic AMP. At 290 mOsm/kg H₂O, addition of 1 mM 8-bromo cyclic AMP increased urea permeability and there was a further increase when osmolality was increased to 490 mOsm/kg H₂O by adding NaCl (Table IV). As with vasopressin, there was no further increase in urea permeability at 690 mOsm/kg H₂O (Table IV). 8-Bromo cyclic AMP (1 mM) and vasopressin (10 nM) stimulated urea permeability similarly at all osmolalities tested. There was no difference in the response of tubules in which osmolality was progressively increased from 290 mOsm/kg H₂O to 690 mOsm/kg H₂O (Fig. 6, solid lines) or decreased from 690 mOsm/kg H₂O to 290 mOsm/kg H₂O (Fig. 6, dashed lines). As with results in the presence of vasopressin, osmolality alone stimulated urea permeability as much as a high concentration of 8-bromo cyclic AMP.


discussion

Our previous work established that urea is transported by a facilitated diffusion pathway in isolated perfused rat terminal IMCDs (2, 3). The present results extend information on this pathway by demonstrating that hypertonic NaCl or mannitol increases urea permeability in the absence of vasopressin and that hypertonic NaCl and vasopressin have additive effects on urea permeability. Osmolality exerts its effect on urea permeability independent of cyclic AMP and appears to act by a mechanism different from that of vasopressin and cyclic AMP. Thus, urea transport in the rat terminal IMCD is regulated by two separate stimuli: vasopressin and inner medullary osmolality and hence, NaCl concentration.

Our previous studies have shown that the urea transporter can function either to reabsorb or secrete urea, depending upon the direction of the imposed urea gradient (2, 7). In the present study, we chose to measure urea transport only in the secretory direction, obviating the need to measure water flux simultaneously (3). We varied perfusate and bath osmolality symmetrically to eliminate transepithelial gradients for water or NaCl movement and hence, the possibility that solvent drag would influence the results. To ensure that the increase in urea perme-

* Data are presented as mean±SE (n = 6). Tubule length, 0.37±0.02. Tubule inner diameter, 24.7±1.8. * These values are significantly different from one another by ANOVA (P < 0.05). † This value is significantly different from 290 mOsm/kg H₂O, but not from 490 mOsm/kg H₂O or 690 mOsm/kg H₂O by ANOVA. Grad urea, log-mean urea gradient; J urea, urea flux; P urea, urea permeability.

![Figure 3](https://example.com/f3.png)  
**Figure 3.** Effect of changing osmolality by adding or removing NaCl in the absence of vasopressin on passive urea permeability in rat terminal IMCDs. Each line connects data from an individual tubule. Solid lines represent tubules in which osmolality was increased. Dotted lines represent tubules in which osmolality was decreased. *P < 0.05 by ANOVA.
Table II. Effect of Changing Osmolality with Mannitol

<table>
<thead>
<tr>
<th>Osmolality (mOsm/kg H2O)</th>
<th>Perfusion Rate</th>
<th>Urea Concentration</th>
<th>Urea Flux</th>
<th>Urea Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nL/m</td>
<td>mM</td>
<td>mM</td>
<td>mM</td>
</tr>
<tr>
<td>290</td>
<td>28.76</td>
<td>0.00</td>
<td>4.91</td>
<td>0.73</td>
</tr>
<tr>
<td>±1.68</td>
<td>±0.00</td>
<td>±0.06</td>
<td>±0.09</td>
<td>±0.07</td>
</tr>
<tr>
<td>490</td>
<td>44.04</td>
<td>0.00</td>
<td>4.90</td>
<td>1.17</td>
</tr>
<tr>
<td>±3.55</td>
<td>±0.00</td>
<td>±0.09</td>
<td>±0.22</td>
<td>±0.20</td>
</tr>
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<td>42.03</td>
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<td>1.39</td>
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<td>±2.87</td>
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<td>±0.13</td>
<td>±0.15</td>
<td>±0.18</td>
</tr>
<tr>
<td>890</td>
<td>43.73</td>
<td>0.00</td>
<td>4.75</td>
<td>1.21</td>
</tr>
<tr>
<td>±2.72</td>
<td>±0.00</td>
<td>±0.12</td>
<td>±0.19</td>
<td>±0.21</td>
</tr>
</tbody>
</table>

Data are presented as mean±SE (n = 6). Tubule length, 0.35±0.03. Tubule inner diameter, 24.7±0.3. * These values are significantly different from 290 mOsm/kg H2O, but not from each other by ANOVA.† This value is significantly different from the urea permeability at 690 mOsm/kg H2O with added NaCl. Grad, log-mean urea gradient; J, urea flux; P, urea permeability.

ability with changes in osmolality was not due to nonspecific effects or osmotic damage to the tubule, osmolality was increased in half of the tubules and decreased in the other half in each experimental protocol. The results were similar regardless of whether osmolality was raised or lowered (Figs. 3–6), indicating that the changes in urea permeability resulted from changes in osmolality. Urea permeability was stable at each osmolality for up to 1 h (Fig. 2) and was independent of perfusion rate (Figs. 1 and 2).

Previously, we reported that adding NaCl to increase osmolality from 290 mOsm/kg H2O to 690 mOsm/kg H2O by adding NaCl resulted in a doubling of urea permeability in the presence of a physiologic concentration of vasopressin (10^{-11} M) (7). The present study extends this result by showing that the stimulus is osmolality rather than NaCl alone. Moreover, the stimulation occurs in the absence of vasopressin and is additive to supraphysiologic concentrations of vasopressin (10^{-8} M). Finally, the effect of osmolality is additive to cyclic AMP.

Cyclic AMP is the second messenger which mediates the effect of vasopressin on urea permeability (11) and osmotic water permeability (12) in rat terminal IMCDs. Hypertonic NaCl and vasopressin have additive effects on adenyl cyclase activity and cyclic AMP production in the inner medulla (13–16). To test whether hypertonic NaCl was increasing urea permeability by increasing cyclic AMP production, we pretreated tubules with 1 mM 8-bromo cyclic AMP. The effect of hypertonic NaCl was additive to that of 8-bromo cyclic AMP on urea permeability, suggesting that the effect of hypertonic NaCl was independent of cyclic AMP. Whether hypertonic NaCl also has a cyclic AMP-dependent effect cannot be determined from the present study.

Studies by Chou et al. (7) have shown that hypertonic urea, added to perfusate and bath, decreases urea permeability in the

![Figure 4](image1.png)  
*Figure 4. Effect of changing osmolality by adding or removing mannitol in the absence of vasopressin on passive urea permeability in rat terminal IMCDs. Each line connects data from an individual tubule. Solid lines represent tubules in which osmolality was increased. Dotted lines represent tubules in which osmolality was decreased. *P < 0.05 by ANOVA.

![Figure 5](image2.png)  
*Figure 5. Effect of changing osmolality by adding or removing NaCl in the presence of 10 nM vasopressin on passive urea permeability in rat terminal IMCDs. Each line connects data from an individual tubule. (Solid lines) Tubules in which osmolality was increased. (Dotted lines) Tubules in which osmolality was decreased. *P < 0.05 by ANOVA.
Table III. Effect of Changing Osmolality with NaCl and 10 nM Vasopressin

<table>
<thead>
<tr>
<th>Osmolarity (mOsm/kg H2O)</th>
<th>AVP (nM)</th>
<th>Perfusion rate (nM)</th>
<th>Urea concentration (mM)</th>
<th>Jurea (pmol/mm/m)</th>
<th>Purea (10^-10 cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>0</td>
<td>35.50</td>
<td>4.84</td>
<td>4.52</td>
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</tr>
<tr>
<td></td>
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<td>4.23</td>
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<td></td>
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<tr>
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<td></td>
<td></td>
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<td>±0.23</td>
</tr>
<tr>
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<td>10</td>
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<td>4.96</td>
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<td></td>
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<td>±0.15</td>
<td>±0.17</td>
<td>±0.25</td>
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</tbody>
</table>

Data are presented as mean±SE (n = 6). Tubule length, 0.27±0.04. Tubule inner diameter, 26.3±2.0. * These values are significantly different from one another by ANOVA (P < 0.05). † This value is significantly different from 290 mOsm/kg H2O without AVP, but not from 290 mOsm/kg H2O with AVP by ANOVA. Gradurea, log-mean urea gradient; Jurea, urea flux; Purea, urea permeability.

The presence of 10^-10 M vasopressin. Thus, effective osmolytes (NaCl, mannitol) stimulate urea permeability, whereas ineffective osmolytes (urea) inhibit urea permeability. Nadler has reported that hyperosmolality increases vasopressin-stimulated osmotic water permeability in rat terminal IMCDs (17). Because atrial natriuretic factor (12) inhibits vasopressin-stimulated osmotic water permeability but has no effect on urea permeability, the results suggest that osmotic water permeability and urea permeability can be regulated independently. The mechanism for differential regulation is not currently known.

The mechanism by which hypertonicity increases urea permeability cannot be determined from these experiments. It cannot be explained by a reduction in cell volume allowing urea to traverse the cell more rapidly, because the apical membrane is rate limiting for transepithelial urea transport (18). A second explanation is that hypertonicity induces a physical change in the conformation of the urea transporter. While stimulation of urea transport by both hypertonic NaCl and mannitol would be consistent with this hypothesis, inhibition of urea transport by hypertonic urea (7) suggests that this explanation is not sufficient. A third mechanism could involve a hypertonicity-induced change in intracellular volume (17) or composition which either activates preexisting transporters or causes their insertion into the membrane. Fourthly, changes in osmolality could initiate synthesis of new transporters, but this seems unlikely given the short time interval (15 min) between changes in osmolality and urea permeability.

Implications for the concentrating mechanism. The production of a concentrated urine depends, in part, upon delivery of urea to the deep inner medulla (1, 8, 9). While previous studies explain how urea reabsorption is delayed until the terminal IMCD (2, 3), they do not provide a mechanism by which urea reabsorption increases within the deepest two-thirds of the inner medulla. Vasopressin-stimulated water reabsorption from the more proximal, urea-impermeable, collecting duct segments concentrates urea within the lumen of the IMCD and establishes a concentration gradient favoring urea reabsorption (2, 3). An increase in interstitial NaCl concentration, resulting from countercurrent multiplication, could be synergistic to vasopressin in progressively increasing urea permeability and

Table IV. Effect of Changing Osmolality with NaCl and 1 mM 8-Bromo Cyclic AMP

<table>
<thead>
<tr>
<th>Osmolarity (mOsm/kg H2O)</th>
<th>cAMP (mM)</th>
<th>Perfusion rate (mM)</th>
<th>Urea concentration (mM)</th>
<th>Jurea (pmol/mm/m)</th>
<th>Purea (10^-10 cm/s)</th>
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<td>±0.04</td>
<td>±0.12</td>
<td>±45.6</td>
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</table>

Data are presented as mean±SE (n = 6). Tubule length, 0.33±0.02. Tubule inner diameter, 24.7±2.7. * These values are significantly different from one another by ANOVA (P < 0.05). † This value is significantly different from 290 mOsm/kg H2O without or with cAMP, but not from 490 mOsm/kg H2O with cAMP by ANOVA. Gradurea, log-mean urea gradient; Jurea, urea flux; Purea, urea permeability.
stimulating urea reabsorption from the terminal IMCD. The increase in interstitial urea concentration would stimulate further NaCl reabsorption from the thin ascending limb, forming a positive-feedback loop for generating the hypertonic inner medulla necessary for antidiuresis. During diuresis, urea reabsorption would decrease because of a lower urea permeability resulting from lack of vasopressin, decreased interstitial NaCl concentration, and a decreased gradient favoring urea reabsorption resulting from lack of vasopressin-stimulated water reabsorption in the cortical collecting duct, outer medullary collecting duct, and initial IMCD.

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References


Figure 6. Effect of changing osmolality by adding or removing NaCl in the presence of 1 mM 8-bromo cyclic AMP on passive urea permeability in rat terminal IMCDs. Each line connects data from an individual tubule. (Solid lines) Tubules in which osmolality was increased. (Dotted lines) Tubules in which osmolality was decreased. *P < 0.05 by ANOVA.