d-Glucose enhancement of water reabsorption in proximal tubule of the rat kidney

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Weinman, Edward J., Wadi N. Suki, and Garabed Eknoyan. d-Glucose enhancement of water reabsorption in proximal tubule of the rat kidney. Am. J. Physiol. 231(3): 777-780. 1976. - Water reabsorption in the proximal convoluted tubule of the rat kidney was examined by in vivo microperfusion techniques in order to examine the effect of d-glucose within the tubular lumen. When tubules were perfused with a balanced artificial solution containing Na, K, Cl, HCO₃, urea, and d-glucose, absolute reabsorption averaged 4.01 ± 0.24 nl/min per mm tubule length. Perfusion with isotonic NaCl resulted in a 37% decrease in reabsorption of 2.54 ± 0.17 nl/min per mm. Addition of d-glucose to the NaCl perfusate enhanced water reabsorption to values similar to those obtained with the balanced artificial perfusate. The enhanced water reabsorption consequent to the addition of d-glucose to the NaCl perfusion solution was completely inhibited by addition of phloridzin to the perfusate. The addition of an unabsorbed hexose, 2-deoxy-d-glucose, to the NaCl perfusate failed to enhance water reabsorption, whereas the addition of an incompletely reabsorbed sugar that is not metabolized, 3-O-methyl-d-glucose, resulted in partial enhancement of the absolute rate of water reabsorption. These studies demonstrate that d-glucose has the specific effect of augmenting water reabsorption in the proximal tubule of the rat kidney.

In a variety of epithelia capable of net water and electrolyte transport, a specific effect of d-glucose on these transport processes has been suggested (7, 8, 11, 14, 18). Indirect evidence has also been advanced for a specific effect of d-glucose on electrolyte and water transport in the kidney of the rat, dog, and man (7, 10, 13, 17, 18). The current studies were designed to directly examine the effect of d-glucose within the tubular lumen on water reabsorption from the proximal convoluted tubule of the rat kidney.

Methods

Studies were performed on male Sprague-Dawley rats weighing 200-300 g with free access to food and water prior to study. Animals were anesthetized and prepared for micropuncture as previously described from this laboratory (21). Surgical fluid losses were replaced with a volume of isotonic saline equal to 1% body wt. An infusion of isotonic saline at a rate of 1.2 ml/h was continued throughout the study.

Proximal convoluted tubules were micropерfused in vivo utilizing a Sage infusion pump (Sage Instruments, Cambridge, Mass.) as previously described (1). [Methoxy-3H]inulin (New England Nuclear Corp., Boston) was added in amounts sufficient to permit a 1% reproducibility in counting to each of the following solutions examined: ultrafiltrates of plasma obtained utilizing Amicon centriflow membrane ultrafilters CF-50 (American Instruments, Lexington, Mass.); a balanced artificial perfusate containing Na+ 139 meq/liter, Cl- 112 meq/liter, K+ 4.5 meq/liter, HCO₃- 30 meq/liter, urea 30 mg/100 ml, and d-glucose 45 mg/100 ml; other perfusion solutions prepared from a 0.9-g/100 ml sodium chloride solution to which one of the following was added: d-glucose (37 or 100 mg/100 ml), 2-deoxy-d-glucose (37 mg/100 ml), 3-O-methyl-d-glucose (37 mg/100 ml), or d-glucose (100 mg/100 ml) plus phloridzin (4.4 mg/100 ml). All solutions were at a pH of 7.4. The osmolality was 285-294 mosmol/kg H₂O.

Following each microperfusion, a latex cast of the tubule was made and the length of perfused tubule determined by microdissection after maceration of the kidney in hydrochloric acid. For each perfusion solution tested, the length of perfused tubule ranged from 0.3 to 2.5 mm with a similar distribution of lengths within each group. Radioactivity of perfusate and collected samples was determined in a modified Bray's solution on a Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). For each perfusion, the following calculations were made: 1) perfusion rate (in nl/min) = CF/PF × collected volume × min⁻¹; 2) absolute reabsorption (in nl/min per mm) = (1 - PF/CF) × perfusion rate × length of perfused tubule in millimeters⁻¹ where CF and PF are the counts per minute in collected perfusate (CF) and perfusion fluid (PF). Statistical significance was determined by the Student t test for unpaired data. All data are expressed as means ± standard error.

Results

Microperfusion of proximal convoluted tubules with ultrafiltrates of plasma resulted in an absolute rate of reabsorption of 3.95 ± 0.31 nl/min per mm (Table 1). Microperfusion with the balanced artificial perfusate resulted in an absolute reabsorptive rate of 4.01 ± 0.24 nl/min per mm, a value not significantly different from that obtained with the plasma ultrafiltrate. Perfusion with the isotonic NaCl solution resulted in a 37% lower...
DISCUSSION

A specific role for d-glucose on the reabsorption of water in the proximal convoluted tubule of the rat is evident from the present experiments. Perfusion with plasma ultrafiltrates or a balanced artificial perfusate (containing Na, K, Cl, HCO₃, urea, and glucose) gave similar results with an absolute rate of reabsorption averaging 4 nl/min per mm. This value is similar to that previously reported from this laboratory and similar to results reported by Buentig and Earley (3, 22) using an identical artificial solution. Perfusion with isotonic sodium chloride alone resulted in a 37% lower rate of absolute reabsorption. The addition of d-glucose in concentrations of 37 or 100 mg/100 ml to the NaCl perfusion solution increased the rate of reabsorption to values similar to those obtained with the balanced artificial perfusate and with the ultrafiltrates of plasma. The specificity of this effect was further examined by addition of phloridzin in a concentration of 4.4 mg/100 ml to the NaCl glucose perfusion fluid. This concentration of phloridzin has been demonstrated to inhibit the net transport of glucose to zero (19), and in the current study it reduced the absolute rate of water reabsorption to values approaching those obtained with NaCl alone.

Glucose has been demonstrated to enhance water and electrolyte transport in studies utilizing isolated perfused kidneys (18, 20). Those studies, however, do not permit a differentiation between the effects of glucose delivered by the peritubular capillaries from the effects of glucose within the tubular lumen. A specific effect of glucose on renal ion transport has also been proposed from studies in which, following the administration of glucose, there is enhanced tubular reabsorption of bicarbonate (17); reduced tubular reabsorption of calcium (10); and, in starving patients, increased reabsorption of sodium (13). These observations, however, have not been adequately explained. Finally, in the isolated proximal tubule of the rabbit and in the proximal tubule of the rat kidney, glucose has been found to be responsible, in part, for the negative electrical potential difference existing in the earlier portions of the proximal tubule (2, 9). This negative potential difference has been interpreted to indicate glucose-stimulated enhancement of sodium reabsorption. In none of these studies, however, were the rates of reabsorption actually measured as was done in the present study. This point becomes critical in view of the recent evidence of Cardinal et al. (5) that the electrical potential difference is not directly related to the rate of water reabsorption.

A considerable body of evidence has been accumulated to indicate that, in a variety of transporting epithelia, glucose enhances the transport of water and electrolytes (7, 8, 14), but the mechanism whereby glucose exerts this effect has not been resolved. In the gut, Schultz and Curran (14) have suggested that there is a common carrier which transports both glucose and sodium across the luminal membrane. The presence of glucose enhancing the affinity of the carrier for sodium and thereby increasing its net rate of reabsorption. By contrast, Fordtran (8) has proposed that the reabsorption of glucose in the gut promotes bulk movement of water and that the major part of the glucose enhancement of sodium reabsorption in the gut is secondary to solvent drag. A similar conclusion for the proximal convoluted tubule of the rat kidney has also been proposed (2, 12). Although definitive resolution of the mechanism by which glucose enhances transport cannot be drawn from the present investigations, certain speculations may be made. If the enhancement of water reabsorption were a consequence of solvent drag, it might be predicted that the rate of water reabsorption would be greater when the rate of glucose reabsorption is increased by raising its concentration in the perfusate from 37 to 100 mg/100 ml (15). As shown in Table 1, the

<table>
<thead>
<tr>
<th>Perfusion Solution</th>
<th>n</th>
<th>PR</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ultrafiltrate</td>
<td>11</td>
<td>20.7 ± 1.7</td>
<td>3.95 ± 0.31</td>
</tr>
<tr>
<td>Balanced artificial perfusate</td>
<td>17</td>
<td>18.6 ± 1.1</td>
<td>4.01 ± 0.24</td>
</tr>
<tr>
<td>NaCl</td>
<td>18</td>
<td>18.9 ± 1.0</td>
<td>2.54 ± 0.17</td>
</tr>
<tr>
<td>NaCl + d-glucose, 37 mg/100 ml</td>
<td>16</td>
<td>16.9 ± 1.4</td>
<td>4.12 ± 0.16</td>
</tr>
<tr>
<td>NaCl + d-glucose, 100 mg/100 ml</td>
<td>14</td>
<td>22.5 ± 1.8</td>
<td>3.85 ± 0.19</td>
</tr>
<tr>
<td>NaCl + 3-O-methyl-d-glucose, 37 mg/100 ml</td>
<td>15</td>
<td>20.8 ± 1.3</td>
<td>2.47 ± 0.19</td>
</tr>
<tr>
<td>NaCl + 2-deoxy-d-glucose, 37 mg/100 ml</td>
<td>16</td>
<td>20.1 ± 1.9</td>
<td>2.44 ± 0.08</td>
</tr>
<tr>
<td>NaCl + 3-O-methyl-d-glucose, 37 mg/100 ml</td>
<td>16</td>
<td>21.1 ± 1.2</td>
<td>3.35 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, number of observations; PR, perfusion rate (in nl/min); C, absolute rate of water reabsorption (in nl/min per mm) of perfused tubule. NaCl perfusion solutions contained 0.9 g/100 ml sodium chloride.

rate of reabsorption of 2.54 ± 0.17 nl/min per mm (P < 0.001 compared to the balanced artificial perfusate). The addition of either 37 or 100 mg/100 ml of d-glucose to the NaCl perfusion solution resulted in higher absolute rates of reabsorption of 4.12 ± 0.16 and 3.95 ± 0.19 nl/min per mm, respectively (P < 0.001 compared to the NaCl perfusion solution).

To further examine the specificity of the ability of glucose to enhance water reabsorption, the proximal tubule was perfused with a solution containing NaCl plus 100 mg/100 ml d-glucose plus phloridzin (4.4 mg/100 ml). Microperfusion with this solution yielded absolute rates of reabsorption of 2.47 ± 0.19 nl/min per mm, a value not different from that obtained with NaCl alone. Microperfusion with a NaCl solution containing the same concentration of phloridzin without glucose at a perfusion rate of 18.7 ± 1.3 nl/min yielded absolute rates of water reabsorption of 2.38 ± 0.38 nl/min per mm (n = 5) (P = NS compared to the NaCl perfusion solution). The addition of 2-deoxy-d-glucose to the NaCl perfusate did not enhance the absolute rate of reabsorption which averaged 2.44 ± 0.08 nl/min per mm, a value not different from that obtained with NaCl alone. The addition of 3-O-methyl d-glucose to the NaCl perfusion resulted in a partial enhancement of reabsorption to 3.35 ± 0.19 nl/min per mm, a value significantly higher than that obtained with the NaCl perfusion solution (P < 0.005) and lower than that obtained with the NaCl plus 37 mg/100 ml d-glucose perfusate (P < 0.001).
rates of water reabsorption at these two concentrations were nearly identical. Furthermore, when the absolute rate of water reabsorption for these two solutions is plotted against the length of perfused tubule (Fig. 1), there is no decrement in the absolute rate of reabsorption with increasing length of perfused tubules, even though the glucose concentration within the lumen must have been variable over a wide range and may be assumed to have fallen to very low levels in the longer perfused segments (15). These findings are at least consistent with the conclusion that solvent drag alone is not an adequate explanation for the effect of glucose on water reabsorption. An alternative explanation may be an alteration by glucose of the permeability of the luminal membrane to sodium as has been demonstrated when amphotericin B is applied to the luminal surface of the proximal tubule of the Necturus kidney (16).

The addition of 3-O-methyl-D-glucose, a sugar that is reabsorbed to a lesser degree than glucose (19), to the NaCl perfusion solution also enhanced water reabsorption but not to the same degree as a comparable concentration of D-glucose. 3-O-methyl-D-glucose is not metabolized by renal tubular cells, and these results would indicate, therefore, that metabolism of the hexose sugar is not necessarily required to enhance water reabsorption (4, 6). The addition of another sugar, 2-deoxy-D-glucose, to the NaCl perfusion solution failed to enhance water reabsorption. Although Woosley et al. (23) have presented indirect evidence that 2-deoxy-D-glucose is reabsorbed by the rat kidney, direct studies on the rat proximal tubule by Ullrich et al. (19) showed no evidence for net transport of this sugar. The failure of 2-deoxy-D-glucose to enhance sodium and water reabsorption and the partial enhancement of 3-O-methyl-D-glucose may be construed as evidence in favor of a requirement for transport of the hexose in order to enhance water reabsorption. On the other hand, if the rate of water reabsorption is not directly related to the rate of hexose transport, as suggested by the studies with D-glucose, the effect of hexoses on water reabsorption must not be simply a consequence of their rates of reabsorption but, rather, a reflection of a more specific effect, perhaps related to certain configurational characteristics of the sugar.

In summary, then, the results of the present investigations confirm that D-glucose enhances water reabsorption in the proximal convoluted tubule of the rat kidney. While speculative, we would interpret the data to indicate that the mechanism of this effect is not solely due to solvent drag and not the consequence of a glucose-sodium carrier system with specific requirements for fixed concentrations of sodium and glucose. The data presented may be interpreted to indicate a specific effect of some hexose sugars, perhaps determined by their structural properties, on the luminal permeability characteristics of the proximal convoluted tubule.

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REFERENCES


