Effects of graded solute diuresis on renal tubular sodium transport in the rat

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A LARGE BODY OF EVIDENCE supports the view that extracellular fluid volume expansion leads to an increase in urinary sodium excretion (2, 5, 7, 8, 11, 17, 24). This natriuresis is largely due to a decrease in proximal tubular sodium reabsorption and the inability of more distally located nephron segments to augment their rate of reabsorption in proportion to the decline in proximal tubular sodium transport. This conclusion is based on direct evaluations of proximal sodium transport by both micropuncture (2, 5, 7, 8, 11, 17, 24) and stationary microperfusion (11, 17, 24) experiments. In contrast, the functional status of the distal nephron during extracellular volume expansion is less well defined and has frequently been inferred only indirectly by comparing late proximal with final urinary excretion rates (2, 7, 8). Also, it is presently not resolved whether the distal sodium transport system can be saturated by 10.220.33.3 on April 29, 2017 http://ajplegacy.physiology.org/ Downloaded from
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The results of the micropuncture experiments have been evaluated by plotting sodium and inulin concentration ratios against tubular length and using the slopes of these relationships to assess the significance of the respective regression lines. Differences between means of excretion rates and of tubular fluid/plasma sodium concentration ratios as well as differences between fractional excretion rates were evaluated by the Student t test. The data are expressed as mean values ± standard error.

Sodium reabsorption was expressed either in terms of fractional transport rates or in absolute terms and calculated by using the observed volume flow rates, sodium, and inulin concentration ratios. Inulin and sodium concentration ratios at 0 and 100% distal tubular length were derived by extrapolation from the mean slopes in the different experimental conditions. This approach has been described in detail in another paper from this laboratory (15).

RESULTS

Table 1 provides a summary of the effects of increasing saline and urea-saline loads on urine flow, glomerular filtration rate (GFR), fractional and absolute excretion rates of water and sodium, and plasma volumes. It is apparent that the effects of increasing either saline or urea-saline loads consisted in a progressive enhancement of urine flow rates, an augmentation of fractional water excretion as evidenced by the progressive fall of urine-to-plasma inulin ratios ((U/P)_In), and an elevation of fractional and absolute excretion rates of sodium. Total-kidney GFR increased slightly. However, this change was not significant. It should be noted that the increments in both fractional and absolute excretion rates of sodium were greater during saline loading than during urea-saline administration. This is illustrated by the smaller increase in fractional and absolute sodium excretion in the latter experimental group. Thus, whereas fractional sodium excretion rates reached peak levels of 9.3% of the filtered load in saline-loaded rats, the urea-saline-infused group excreted only 4.7% of the filtered sodium load at the highest infusion rates. Similarly, at a given infusion rate, the absolute rate of sodium excretion is significantly higher in the sodium chloride-loaded group. Related to the more powerful natriuretic effect of sodium chloride infusion is the observation that the urinary sodium concentration is markedly elevated above plasma levels ([U/P]Na > 1.0), whereas these ratios never exceed mean values of 0.5 even at the highest rates of urea-saline diuresis. Plasma volumes as estimated from the fall in hematocrit

<table>
<thead>
<tr>
<th>Flow Rates</th>
<th>VUR, ml/min/kg</th>
<th>GFR, ml/min/kg</th>
<th>(U/P)_In</th>
<th>(U/P)_Na</th>
<th>UNa × VUR, mcg/min/kg</th>
<th>Plasma Vol, A%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0.0233 ± 0.0026</td>
<td>8.43 ± 0.93</td>
<td>42.8 ± 46.3</td>
<td>0.621 ± 0.137</td>
<td>2.846 ± 0.772</td>
<td>14.96 ± 2.63</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.311 ± 0.050</td>
<td>9.27 ± 0.86</td>
<td>45.7 ± 10.9</td>
<td>1.597 ± 0.178</td>
<td>65.62 ± 9.42</td>
<td>35.09 ± 3.45</td>
</tr>
<tr>
<td>High</td>
<td>0.583 ± 0.073</td>
<td>10.03 ± 1.10</td>
<td>17.0 ± 5.1</td>
<td>1.409 ± 0.138</td>
<td>106.5 ± 11.2</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.0653 ± 0.0110</td>
<td>9.12 ± 1.60</td>
<td>187.7 ± 43.1</td>
<td>0.305 ± 0.086</td>
<td>2.259 ± 0.584</td>
<td>17.45 ± 1.15</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.2391 ± 0.0534</td>
<td>9.39 ± 1.06</td>
<td>49.4 ± 7.9</td>
<td>0.325 ± 0.063</td>
<td>7.543 ± 2.838</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.8836 ± 0.1310</td>
<td>10.11 ± 1.16</td>
<td>13.9 ± 2.1</td>
<td>0.495 ± 0.072</td>
<td>55.924 ± 8.039</td>
<td>22.75 ± 2.25</td>
</tr>
</tbody>
</table>

Values are means ± SE.
increased significantly over control values during the infusion of both saline and urea-saline solutions, the increase being larger in the group of animals receiving saline loads.

**Effects on proximal tubular functions.** Table 2 summarizes the effects of the different experimental procedures on proximal tubular fluid and sodium transport. Confirming previous observations from our (17) and other (2, 5, 7, 8, 11, 24) laboratories, we found that intravenous saline loading resulted in a marked depression of fractional fluid and sodium reabsorption. Single-nephron GFR and tubular flow rate were also enhanced with the induction of diuresis. It was only at the highest infusion rates of urea-saline solutions that the tubular sodium concentration fell significantly from a mean value of 141 to 128 meq/liter ($P < 0.005$).

**Effects on distal tubular functions.** Graphical summaries of our results on distal tubular sodium reabsorption under control conditions and various levels of saline diuresis are shown in Fig. 1. TF/P sodium and TF/P sodium-to-inulin ratios (TF/P)$_{Na}$ (TF/P)$_{Na}/$I$_{In}$ are plotted against distal tubular length. Considering first the progression of sodium concentration ratios, it is apparent that under conditions of lowest urine flow rate there is a significant decline of (TF/P)$_{Na}$ from 0.55 to 0.26 at 0 and 100% distal tubular length along the distal tubule. This decline is attenuated at moderate flow rates ((TF/P)$_{Na}$ of 0.75 and 0.44 at 0 and 100% distal tubular length) and abolished at the highest tubular flow rates. We conclude that an increase in delivery of fluid from the loop of Henle into the distal tubule leads to an increase in distal tubular sodium concentration. The elevation of tubular sodium concentration occurs mainly along the second half of the distal tubule, whereas the sodium concentration at the beginning of the distal tubule is not greatly altered.

The finding of an elevation of distal tubular sodium concentration ratios with the augmentation of distal tubular flow rate is further supported by consideration of our distal tubular recollection data. The mean experimental/control sodium recollection ratio during moderate diuresis was 1.47 ± 0.15 ($P < 0.05$); that during high flow rates was 2.52 ± 0.30 ($P < 0.01$).

As summarized in Fig. 2, similar considerations of distal tubular data during urea-saline infusions show a smaller rise of tubular sodium concentrations, no doubt due to the progressive accumulation of urea, a poorly permeant solute, in the late distal tubule, a nephron site characterized by its low urea permeability. Thus, the respective (TF/P)$_{Na}$ ratios extrapolated to 0 and 100% distal tubular length were 0.47 and 0.17 during low, 0.48 and 0.18 during moderate, and 0.52 and 0.35 during high urine flow rate.

**Table 2. Summary of end-proximal micropuncture data**

<table>
<thead>
<tr>
<th>Flow Rates</th>
<th>(TF/P)$_{Na}$</th>
<th>% Vol Tubular Flow, GFR/Nep</th>
<th>Tubular Flow, GFR/Nep</th>
<th>(TF/P)$<em>{Na}/$I$</em>{In}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline diuresis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2.00 ± 0.09</td>
<td>46.6 ± 2.5</td>
<td>13.14 ± 0.93</td>
<td>29.7 ± 2.8</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.50 ± 0.06</td>
<td>31.8 ± 2.9</td>
<td>49.99 ± 1.79</td>
<td>45.3 ± 3.3</td>
</tr>
<tr>
<td>High</td>
<td>1.21 ± 0.05</td>
<td>16.6 ± 2.6</td>
<td>56.78 ± 2.35</td>
<td>64.8 ± 2.2</td>
</tr>
<tr>
<td>Urea-saline diuresis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2.06 ± 0.11</td>
<td>49.4 ± 2.4</td>
<td>14.01 ± 0.49</td>
<td>29.1 ± 1.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.51 ± 0.05</td>
<td>33.1 ± 2.2</td>
<td>20.44 ± 1.42</td>
<td>36.3 ± 2.4</td>
</tr>
<tr>
<td>High</td>
<td>1.34 ± 0.05</td>
<td>20.4 ± 1.4</td>
<td>41.55 ± 5.46</td>
<td>50.7 ± 6.2</td>
</tr>
</tbody>
</table>

Values are means ± SE.
mean experimental/recollection (TF/P)\textsubscript{Na} ratio was significantly elevated only during high flow rates (1.90 ± 0.32, \(P < 0.05\)).

Table 3 summarizes the mean fractional reabsorption rates of sodium along different nephron segments. It is apparent that the fractional reabsorption of sodium along the proximal convolution is depressed following both saline and urea-saline loading. In sharp contrast, we have not observed a similar decline of reabsorptive transport along the more distally located parts of the nephron. Thus, fractional reabsorption rate remained constant or increased with the delivery of larger fractions of the filtrate into the loop of Henle or into the distal convoluted tubule. With the exception of the highest rate of urea-saline infusions in which the fractional rate of sodium reabsorption along the collecting duct rose, there was no major change in fractional sodium reabsorption rate across the collecting duct epithelium. We conclude from these results that the sodium transport system of the proximal convolution responds to an increase in the filtered load during extracellular volume expansion with a sharp fall of net sodium transport. This confirms findings of previous studies (2, 5, 7, 8, 11, 17, 24).

Figure 3 presents a graphic summary of absolute tubular reabsorption rates, normalized to total distal tubular length, as a function of distal tubular flow rate. It is clear that distal tubular reabsorption increases linearly with flow rate in both saline and urea-saline-loaded animals. An increase in absolute rates of distal tubular sodium reabsorption along the distal convolution of the rat tubule after enhancement of distal sodium delivery has also been reported by Kunau (16). In our experiments, the stimulation of sodium reabsorption with augmented flow rate is less marked in animals receiving the urea-saline load. During neither saline nor urea-saline loading did we, even at the highest flow rates, observe evidence of saturation of the sodium transport system. As expected, this functional behavior leads to a linear proportionality between the sodium loading entering the distal tubule and the rate of
associated with a significant increase in distal tubular sodium reabsorption. This enhancement of sodium transfer is closely associated with a significant increase of sodium and fluid reabsorption. The more powerful stimulation of sodium reabsorption by saline loading or loading with a urea-saline solution summarizes relevant data from our saline loading experiments.

Data from animals receiving isotonic saline or urea-saline loads at progressively increasing rates.

We conclude from the data presented that the extent to which saline loading or loading with a urea-saline solution increases distal tubular sodium reabsorption depends strongly on the effectiveness of these maneuvers to raise the tubular concentration of sodium ions (see Fig. 1, 2, and 3). The more powerful stimulation of sodium reabsorption by saline administration as compared to that of urea-saline is clearly associated with a greater rise of the distal tubular sodium concentration during saline loading.

**DISCUSSION**

Our experiments demonstrate that the distal tubular epithelium of the rat responds to the increased delivery of fluid with a significant increase of sodium and fluid reabsorption. This enhancement of sodium transfer is closely associated with a significant increase in distal tubular sodium concentrations as larger fractions of the glomerular filtrate enter the distal tubule. The functional behavior of the distal tubule is in sharp contrast to the proximal tubule, a nephron site that responds to extracellular volume expansion and increased tubular flow rates with a marked fall of sodium and fluid transport subsequent to volume expansion, such as dilution of peritubular proteins and an increase in peritubular hydrostatic pressure (3, 20, 25, 26), are less effective or ineffective at the level of the distal tubule (1). The present experiments do not permit a critical assessment of the effects of volume expansion per se upon the intrinsic properties of the distal tubular sodium transport system, since we have not compared the handling of similarly increased distal tubular sodium loads in animals which were not volume expanded. Conceivably they could have responded to the sharply increased delivery of fluid with an even larger increment of sodium reabsorption. Morgan and Berliner (23) observed in experiments in which they pump perfused single distal tubules in normovolemic and volume-expanded rats that net sodium reabsorption remained unchanged. They concluded that the distal tubular response to increased fluid and sodium delivery is not altered during volume expansion.

The results of the present series of experiments indicate that the significant augmentation of distal tubular sodium reabsorption is accompanied by a redistribution of the sodium load along the distal tubule. Normally, the sodium concentration declines along the distal tubule and reaches low values by the end of this nephron segment. These concentrations fall into the range achieved in steady-state conditions such as raffinose-split-drop experiments (13, 14, 22). Associated with this fall in sodium concentration along the distal tubule progressively less sodium is reabsorbed (10).

Thus, under hydropenic, nonvolume-expanded conditions, most of the sodium load entering the distal tubule is reabsorbed early along the distal tubule, and the later parts of the distal tubule are exposed to a range of sodium concentrations which are quite low and correspond to maximal and thus limiting transepithelial concentration differences. With large increments of fluid emerging from the loop of Henle after volume expansion, the early part of the distal tubule fails to lower the sodium concentration as effectively as in normovolemic conditions. Hence, later parts of the distal tubule are exposed to fluid of increasing sodium concentrations. The fact that they respond to this load increment with a sharp enhancement of reabsorption indicates that they are normally operative well below saturation levels. Similar to the results of microperfusion studies (23), we have not observed evidence in support of a saturable sodium transport system (distal Tm), since the relationship of distal tubular sodium reabsorption to flow rate in both saline- and urea-saline-loaded animals remained linear over more than a 10-fold range of flow increments.

The marked change of the sodium concentration profile and the subsequent redistribution of sodium reabsorption along the distal tubule are likely to be related to two factors. We have demonstrated previously that the magnitude of the maximal transepithelial sodium concentration differences across the distal tubule, which can be achieved in

**FIG. 3.** Plot of absolute rates of normalized (0-100%) distal tubular sodium reabsorption as function of distal tubular volume flow rate. Data from rats receiving isotonic saline or urea-saline loads at progressively increasing rates.

**FIG. 4.** Plot of absolute rates of normalized (0-100%) distal tubular sodium reabsorption as function of sodium load entering distal tubule. Data from animals receiving isotonic saline loads at progressively increasing rates.
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steady-state split-drop experiments using raffinose, is not affected by massive extracellular volume expansion with saline (17). Hence, the higher sodium concentrations along the distal tubule during saline diuresis are not due to inhibition of the distal tubular sodium pump. Rather, we believe that they are related to a shortening of distal tubular contact time and changes in urea recirculation. Both of these occur during saline diuresis and are likely to affect the properties of the distal tubular sodium transport system.

The induction of a diuresis by the administration of both saline or urea-saline loads leads to the reduction of distal tubular contact time. Estimated from the time of appearance and disappearance of lissamine green in individual distal tubules, the contact time of fluid decreased from a mean control value of 44.2 ± 2.4 to 32.2 ± 2.9 s during saline diuresis and from a mean value of 56.4 ± 3.1 to 32.3 ± 2.7 s during urea-saline diuresis. These changes are significant (saline diuresis P = <0.05), urea-saline diuresis P = <0.03). It is possible that the shortening of the contact time of fluid at the early distal tubular level prevents the attainment of normal transepithelial concentration differences and leads to a progressive rise of sodium concentrations. Most split-drop experiments dealing with the study of the magnitude of distal tubular sodium concentration differences have been done after some 40–60 s had been permitted to elapse (12–14, 22). Precise data on the development of transtubular sodium concentration differences over shorter time periods are not available. However, the magnitude of the shortening of the contact time in the diuretic states of the present study is in the range (<1 min) which could conceivably lower the distal tubular sodium concentration. With respect to the modifications of sodium transport at the later part of the distal tubule, two opposing factors are operative. One, a decrease in contact time, would tend to lower sodium transport by exposing the tubular fluid to the distal tubular epithelium for a shorter than normal time period; the second factor would be the increase in sodium concentration. This would augment the reabsorptive transport rate by elevating the substrate level of the sodium pump. Of the two factors the second one clearly dominates in view of the observed significant enhancement of overall distal tubular sodium transport.

A second factor which must be considered is that of urea accumulation along the distal tubule. Lassiter et al. (18, 19) and Danielson et al. (6) have provided evidence that an important fraction of urea entering the collecting ducts reenters the loop of Henle and is concentrated along the distal tubules. The latter are distinguished by low urea permeability (4). These investigators also demonstrated a strong flow dependence of the process of urea-recirculation such that urea reentry into the distal tubule fell sharply with increased urine flow rates. Since late distal tubular fluid in the rat approaches isotonicity, it is clear that its urea content affects the extent to which the sodium concentration can increase with flow augmentation. At low tubular flow rates, urea recirculation leads to accumulation of this poorly permeant solute at the late distal tubular level and accentuates the fall in sodium concentration similar to the action of mannitol in the proximal tubule (13, 14, 26).

With the increase in flow rates, smaller amounts of urea recirculate and a progressively larger moiety of the late distal tubular solute load will be made up by sodium ions. Clearly, the extent to which the distal tubular sodium concentration rises with flow rate is affected by the presence of nonsodium solutes of which urea normally represents the largest fraction.

The relationship between late distal tubular transepithelial sodium concentration differences and urea content is further underscored by the present observation that the infusion of a urea-saline load compromises the increase of sodium concentration which results from the escape of larger than normal fluid loads from Henle’s loop. Whereas the mean end-distal (1F/P)Na increased to 0.80 in saline-loaded rats, a smaller increase to 0.35 was observed in urea-saline loaded animals at similar rates of distal tubular flow. It is clear from the above discussion that the presence of urea significantly modifies the response of the distal tubule to increased flow rates.

In summary, the present study provides evidence that the distal tubule responds to an overload of sodium with a sharp increase in net sodium reabsorption. The transport stimulation is accompanied by a redistribution of the distal sodium load to normally unsaturated late distal tubular segments. Associated with this redistribution is a prominent rise in sodium concentrations which appears to be fundamental to the enhancement of sodium reabsorption. Evidence is also presented that urea importantly modulates the relationship between the augmentation of distal flow, the distal tubular sodium concentration profile, and the net rate of sodium reabsorption.

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