Renal blood flow, early distal sodium, and plasma renin concentrations during osmotic diuresis

PAUL P. LEYSACC,1 NIELS-HENRIK HOLSTEIN-RATHLOU,1 AND OLE SKØTT2
1Department of Medical Physiology, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen; and 2Physiology and Pharmacology, University of Southern Denmark, Odense University, DK-5000 Odense C, Denmark

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Leyssac, Paul P., Niels-Henrik Holstein-Rathlou, and Ole Skott. Renal blood flow, early distal sodium, and plasma renin concentrations during osmotic diuresis. Am J Physiol Regulatory Integrative Comp Physiol 279: R1268–R1276, 2000.—Inconsistencies in previous reports regarding changes in early distal NaCl concentration (EDNaCl) and renin secretion during osmotic diuresis motivated our reinvestigation. After intravenous infusion of 10% mannitol, EDNaCl fell from 42.6 to 34.2 mM. Proximal tubular pressure increased by 12.6 mmHg. Urine flow increased 10-fold, and sodium excretion increased by 177%. Plasma renin concentration (PRC) increased by 58%. Renal blood flow and glomerular filtration rate decreased, however end-proximal flow remained unchanged. After a similar volume of hypotonic glucose (152 mM), EDNaCl increased by 3.6 mM, (P < 0.01) without changes in renal hemodynamics, urine flow, sodium excretion rate, or PRC. Infusion of 300 μmol NaCl in a smaller volume caused EDNaCl to increase by 6.4 mM without significant changes in PRC. Urine flow and sodium excretion increased significantly. There was a significant inverse relationship between superficial nephron EDNaCl and PRC. We conclude that EDNa decreases during osmotic diuresis, suggesting that the increase in PRC was mediated by the macula densa. The results suggest that the natriuresis during osmotic diuresis (8, 11, 20). However, an early study from Gottschalk’s laboratory (29) had shown a decrease in (TF/P)Na from 0.62 in hypotonic glucose to 0.24 during osmotic mannitol diuresis. Similarly, Churchill et al. (4) found a decrease in distal TFNa but an increase in distal sodium load during massive mannitol diuresis. The reason for these apparent inconsistencies remains unclear, and the contribution of the inhibition of sodium reabsorption in the proximal tubule and Henle loop to the natriuresis has not been finally settled.

In rats, infusion of hypertonic mannitol increased renal plasma flow (RPF), whereas renal blood flow (RBF) remained either unchanged (2) or increased slightly (3) due to the decrease in hematocrit (Hct) and possibly afferent vasodilation. The vasodilation is associated with an increase in medullary blood flow that is assumed to cause a washout of the medullary hypertonicity (3). This, in turn, reduces water reabsorption in the tDLH and the collecting ducts and thereby the ability to concentrate the urine, which would explain the marked diuresis.

The effects on glomerular filtration rate (GFR) have been less consistent. Most studies report a decrease in GFR after hypertonic mannitol (1, 10, 15, 16, 23), but the opposite has also been reported, at least in superficial nephrons (2). Viewed together, the decrease in GFR is only modest. This is surprising in view of the marked increases in Pprox reported (2, 10, 16). Therefore, glomerular capillary pressure must have increased almost parallel with the tubular pressure, suggesting efferent arteriolar vasoconstriction and/or afferent vasodilatation.

The mechanism of the apparent mannitol-induced efferent arteriolar vasoconstriction and/or afferent va-
sodilation in rats is unexplained, because one would expect that the observed increased ED\textsubscript{NaCl} after mannitol diuresis would activate the tubuloglomerular feedback mechanism (TGF) thereby causing afferent vasoconstriction rather than dilatation (22); and it should depress renin release causing efferent relaxation (22). On the other hand, Churchill et al. (4) reported that renin secretion and plasma renin activity were depressed to the same degree in saline and mannitol diuresis despite an increase in ED\textsubscript{NaCl} after saline and a decrease after mannitol.

These apparent inconsistencies motivated us to measure ED\textsubscript{NaCl} during mannitol diuresis in rats. In addition, we measured P\textsubscript{prox}, GFR, end-proximal flow rate, RBF, and plasma renin concentrations (PRC). One group of control animals received glucose to produce the same degree of volume expansion, and another group was infused with NaCl to increase the sodium excretion without significant volume expansion.

METHODS

Animal Preparation

Male Sprague-Dawley rats (Møllegaard’s breeding center, L1.Skensved, Denmark), 245–330 g body wt, were allowed free access to water and Altromin standard rat pellets with a sodium content of 87 mmol/kg dry weight of food. In series, including clearance measurements, the diet given 2 days before the experiment was a wet-mash diet containing (in mmol/kg dry weight of food) 220 sodium, 220 potassium, and 15 or 20 lithium (as LiCl). This gave plasma lithium concentrations ranging from 0.15 to 0.30 mM. Anesthesia was induced with 5% halothane delivered in a mixture of 35% oxygen and 65% nitrogen from a Fluotec Mark-3 vaporizer. Polyethylene catheters were inserted into the left jugular vein for infusions and into the right carotid artery for continuous recording of the systemic arterial blood pressure. After tracheostomy, the rats were placed on a servo-controlled heated operating table that maintained their body temperature at 37°C. The rats were connected to a small-rabbit ventilator, adjusted to maintain arterial plasma pH between 7.35 and 7.45, with a mixture of 35% oxygen and 65% nitrogen, tidal volume 1.9–2.1 ml, and a frequency of 60 min. The final halothane concentration necessary to maintain adequate anesthesia was ~1%. After an intravenous priming dose of 6 mg gallamine triethiodide (Relaxon) in 0.6 ml of 0.9% saline, a continuous intravenous infusion of 60 mg gallamine triethiodide in 5 ml of 0.9% saline was given at 20 μl/min. The rats were prepared for micropuncture as previously described in detail (26). In brief, the left kidney was exposed through a midline incision extended to the flank, and the left ureter was cannulated for urine collections. The left kidney was immobilized with a lucite ring and superfused otherwise described in detail (26). In brief, the left kidney was exposed through a midline incision extended to the flank, and the left ureter was cannulated for urine collections. The left kidney was immobilized with a lucite ring and superfused with saline preheated to 37°C. The renal capsule was left intact.

Hydrostatic Pressure Measurements

Systemic arterial pressures were measured by a Statham P23Db pressure transducer (Gould, Oxnaard, CA). Intratubular hydrostatic pressures were measured by a servo-nulling micropressure system, manufactured according to the description of Intaglialetta et al. (9) by Baumbach Electronics (Copenhagen, Denmark), and connected to a Statham P23Db transducer. The sharpened pressure pipettes (1- to 2-μm OD) were filled with 1 M NaCl solution colored with Lissamine green (0.6 g/100 ml). The system was calibrated each day in a small pressure chamber. It was linear over the range 0–100 mmHg and permitted pressures to be recorded within ±0.5 mmHg. All recordings of pressures were recorded on a Goertz Servorgor recorder (Vienna, Austria) and simultaneously on a frequency-modulated (FM) tape recorder.

TF Electrical Conductivity

Electrical conductivity of ED-TF was measured in situ by the microprobe method of Gutsche et al. (7). The microprobe was made from micropipettes pulled from 0.9-mm (OD) Pyrex glass capillaries with filament (Clark Electromedical, Pangbourne, England). The beveled tip was 6–8 μm (OD). The outer surface of the pipette was covered with platinum by applying a liquid platinum-salt resin (Glanzplatin, Degussa, Frankfurt, Germany) and dried at 180°C. The pipette was filled with isotonic saline, and a platinum wire was inserted into the pipette shank. The pipette holder was adapted to a microperfusion and suction pump (Fa. Hampel, Frankfurt, Germany). The electrical connection between the external platinum coat of the pipette and its lead was provided by conductive silver paint (Leitsilber, Degussa). The leads from both electrodes were connected to an alternating-current (AC) voltage generator and a linear AC amplifier (Baumbach Electronics). The output was recorded on the Goertz Servorgor recorder and FM tape recorder. For measurements, fluid was continuously aspirated at a low rate (6 nl/min) into the tip of the pipette thus providing electrical contact between the two electrodes. A constant AC voltage across the two electrodes generates an AC proportional to the conductivity of the aspirated fluid that is expressed in terms of millimoles of NaCl. The saline solution bathing the kidney surface served as a reference before and after each measurement. A calibration curve made from serial dilutions of a 154 mM NaCl solution gave a linear relation between the measured conductivity and corresponding NaCl concentration measured with flame emission photometry over a range from 15 to 154 mM (17).

RBF Recordings

RBF was measured continuously with a sinus-wave electromagnetic blood flow meter (Scalar Medical, model 1402, Delft, Netherlands). A perivascular flow sensor (lumen diameter 0.7 or 0.8 mm) was placed on the left renal artery. The flow meter was calibrated each day. The output was recorded on the Goertz Servorgor recorder and FM tape recorder.

Experimental Protocol

One group was given infusions of 10% mannitol (group I). A control group (group II) was given infusions of hypotonic glucose (152 mM) to achieve a similar intravenous NaCl-free volume load. Otherwise, the protocol was identical in the two groups. A third group (group III) was given a physiological sodium load (10 μmol/min NaCl iv for 30 min) to compare the changes in ED-NaCl delivery, Na excretion, and PRC with those in group I.

Group I

Series Ia (n = 14). After an equilibration period, a proximal tubule was impaled with a pressure-recording micropipette, and the corresponding ED tube identified by injection of a small amount of Lissamine green colored fluid from the pressure pipette. P\textsubscript{prox} was recorded continuously throughout the experiment. The conductivity pipette was inserted in the...
earliest possible visible distal convolution. A first arterial blood sample (~250 µl) for measurements of plasma sodium (PNa) and PRC was taken from the carotid catheter. When recordings of mean arterial pressure (MAP), P_prox, and conductance had stabilized, urine was collected, and continuous recordings of pressures and conductivity were obtained during a 5- to 10-min control period and were continued during the rest of the experiment. After the control period, an intravenous infusion of 10% mannitol was started at 350 µl/min for 5 min followed by a continuous infusion at 150 µl/min throughout the experiment. After 20 min of mannitol loading [total dose 0.4 g equal to 1.54 g (kg body wt)], a second urine sample was collected.

Series Ib (n = 8). For clearance determinations, [51Cr]EDTA (Hoechst-Behring, Frankfurt, Germany) was given intravenously as a priming dose of 13.77 µCi (0.51 MBq) in 1.75 ml of saline followed by a continuous intravenous infusion of 0.158 µCi (0.0658 MBq/min) in 20 µl/min. The experiment started after an equilibration period of ~60 min. During the equilibration period, the renal artery was dissected free from perivascular connective tissue, and the electromagnetic blood flow probe was placed on the artery. RBF was measured continuously throughout the experiment. After the first blood sample, two urine collections (each 5 to 10 min) for the control clearance period were obtained. A second blood sample was taken at the end of the last urine collection. An intravenous infusion of 10% mannitol was then started at 350 µl/min for 5 min followed by a continuous infusion at 150 µl/min throughout the experiment as in series Ia. After 20 min of mannitol loading, blood and urine samples were obtained as above for the experimental clearance period. At the end of the experiment, the kidney was removed, drained, and weighed.

Series Ic (n = 8). The results of series Ib showed that the mannitol infusion caused ED_NaCl to decrease to a minimum reached within 3–6 min followed by a gradual modest increase over the next ~20 min to a value still below the control value. Therefore, an additional, but otherwise similar, series was performed in which P_prox and ED conductance were measured before and during the 10% mannitol infusion at 350 µl/min and for 20 min of the mannitol infusion at 150 µl/min. After the 20-min mannitol infusion, a second blood and urine sample was collected while the infusion was continued for an additional 40–45 min. At the end of the 60-min infusion period, the same proximal and ED convolutions were repunctured, and recordings of P_prox and ED conductance were repeated. A final (third) urine and blood sample was collected at the end of the experiment.

Group II

Series Ia (n = 8) and Ib (n = 8). The mannitol solution was replaced by hypotonic glucose. Otherwise, the protocols were identical to the Ia and Ib series of group I.

Group III

The protocol was similar to the Ia series of group I, except that isotonic NaCl solution was given instead of mannitol at a rate of 65 µl/min for 30 min, corresponding to a total load of 300 µmol of Na (n = 11).

Analytic Methods

[54Cr]EDTA activity was measured by a SELECTRONIC well-scintillation counter (Molsgaard Medical, model 54–23, Copenhagen, Denmark). Lithium in plasma and urine was measured by atomic absorption spectrophotometry (Perkin Elmer 2380). Sodium and potassium in plasma and urine were measured by flame-emission photometry (Perkin Elmer 2380). Urine flow was measured gravimetrically. PRC was measured by the ultramicroradiommoassay of generated ANG I with the antibody-“trapping” technique of Lykkegaard and Poulsen (19). Aliquots of plasma were diluted 20- to 80-fold with Tris buffer containing human albumin, and 5-µl portions of these samples were incubated for 24 h at 37°C with 20 ml of a reaction mixture that contained purified rat renin substrate (–1,200 ng ANG I-equivalents/ml). This incubation was followed by radioimmunoassay of generated ANG I. PRC was measured in reference to renin standards obtained from the National Institute for Biological Standards and Control (Potters Bar, Herts, UK; 1 milli Goldblatt unit = 160 pg ANG I·ml⁻¹·h⁻¹). Blood glucose was measured with a “One Touch” apparatus (Lifescan, Johnson & Johnson, Milpitas, CA), and glucosuria was estimated with BMS-test-5L sticks (Boehringer Mannheim).

Calculations. Concentrations of lithium and of [54Cr]EDTA were calculated according to the conventional expression:

\[ C_i = \frac{[U/P]_i \times (V/U)}{K_i} \]

where \([U/P]_i\) denotes the urine-to-plasma concentration ratio of the substance \(x\), and \((V/U)/K_i\) denotes the rate of urine flow divided by the kidney weight. The plasma concentration corresponding to the midpoint of each clearance period was estimated by interpolation from the curve relating the measured plasma concentrations to the time of collection. [54Cr]EDTA clearance has been validated as a reliable measure of total kidney and single-nephron GFR in the rat (14).

Because the animals were in sodium balance on a normal sodium intake well above that causing distal lithium reabsorption (27), C_Li gives a good estimate of water flow rate at the end of the proximal straight segment (13, 26). Proximal fractional reabsorption of fluid may then be calculated as:

\[ RRF = \frac{C_{L_i}}{C_{GFR}} \]

where \(C_{GFR}\) is the GFR of a representative experiment is shown in Fig. 1. Continued mannitol infusion for additional 40–45 min was not followed by any further increase in ED_NaCl. The mean

RESULTS

The data are given as mean values ± SE in Tables 1 and 2. Body weight and control MAP were similar in all groups and did not change during the experimental periods. The micropuncture series a and c show that ED_NaCl decreases after mannitol infusion, initially to a minimum of 27 mM reached after 4–5 min followed by a moderate increase to a value of ~34 mM after 20 min, still significantly lower (~8.4 mM, \(P < 0.001\)) than the control value of ~43 mM. Because the values before mannitol administration and during the 20-min infusion were similar in series Ia and Ic, the data were pooled and given in Table 1. The trace from a representative experiment is shown in Fig. 1. Continued mannitol infusion for additional 40–45 min was not followed by any further increase in ED_NaCl. The mean...
value of ~32 mM at 60 min was significantly lower than the control value ($P < 0.005$), but it was not statistically different from either the initial value at 5 min or the value reached after 20 min ($n = 8$). The scattergram (Fig. 2A) shows the individual changes in ED$_{NaCl}$. P$_{prox}$ increased markedly after mannitol, initially to ~35 mmHg, and was 12.6 mmHg above the normal control value of 13.7 mmHg after 20 min. Urine flow increased ~10-fold, whereas sodium excretion rate increased by ~60%. P$_{Na}$ concentration increased significantly (~58%). The tendency of a decrease in P$_{Na}$ concentration was statistically insignificant (Table 1).

The same volume load with hypotonic glucose solution caused a modest but consistent increase in ED$_{NaCl}$ from ~40 to 45 mM ($P < 0.01$) (Fig. 2B), whereas P$_{prox}$ increased by 1 mmHg. The trace from a representative experiment is shown in Fig. 3. Neither urine flow nor sodium excretion changed significantly, but P$_{Na}$ concentration decreased by ~7 mM ($P < 0.01$). PRC remained unchanged. Plasma glucose concentration increased from 7.6 to 11 mM, which caused moderate glucosuria in some but not all experiments, indicating that the plasma concentration reached was close to the threshold value (Table 1).

The sodium load with a smaller volume load caused an increase in ED$_{NaCl}$ from ~42 to 48 mM ($P < 0.001$) without any change in P$_{prox}$. Urine flow increased moderately, and sodium excretion rate doubled. P$_{Na}$ concentration remained unchanged. Plasma potassium concentration decreased slightly but significantly. PRC tended to decrease, but the change was not significant (Table 1).

Figure 4 shows PRC as a function of ED$_{NaCl}$. To obtain a sufficient range of ED$_{NaCl}$ control and 20-min values (separate symbols) from both the mannitol group I and group III, given the sodium load, are included. The results show a significant ($P < 0.01$) inverse relationship over the physiological range of ED$_{NaCl}$ from 22 to 56 mM. As shown in Fig. 5, there was no significant relationship between the change in PRC and the change in sodium excretion after mannitol (A), whereas sodium excretion rate increased almost hyperbolically with decreasing PRC after the sodium load (B).

Table 1. The microdilution series a and c

<table>
<thead>
<tr>
<th></th>
<th>Group I, Mannitol</th>
<th>Group II, Glucose</th>
<th>Group III, NaCl</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Initial</td>
<td>After</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>277±4.6</td>
<td>279±8.8</td>
<td>275±5.2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>112±2</td>
<td>115±2</td>
<td>112±3</td>
</tr>
<tr>
<td>P$_{prox}$, mmHg</td>
<td>13.7±0.3</td>
<td>13.0±0.4</td>
<td>13.1±0.5</td>
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<tr>
<td>ED$_{Na}$, mM</td>
<td>42±0.6</td>
<td>41.2±2.4</td>
<td>45.8±2.1</td>
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<tr>
<td>V$_{ur}$ μl/min</td>
<td>6.8±0.5</td>
<td>7.4±3.6</td>
<td>67.7±4.6</td>
</tr>
<tr>
<td>Na$_{excr}$, nmol/min</td>
<td>799±135</td>
<td>2,215±491</td>
<td>1,416±484</td>
</tr>
<tr>
<td>PRC, ×10$^{-5}$ GU/ml</td>
<td>4.58±4.8</td>
<td>7.65±9.4</td>
<td>28.0±9.5</td>
</tr>
<tr>
<td>P$_{Na}$, mM</td>
<td>143±2</td>
<td>143±1</td>
<td>136±1</td>
</tr>
<tr>
<td>P$_{K}$, mM</td>
<td>4.7±0.2</td>
<td>4.8±0.2</td>
<td>0.1±0.15NS</td>
</tr>
<tr>
<td>P$_{glucose}$, mM</td>
<td>7.6±0.3</td>
<td>11.0±0.7</td>
<td>3.4±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ (paired t-test); NS, nonsignificant. §In this series, $n = 6$ because of analytic problems. MAP, mean arterial pressure; P$_{prox}$, proximal tubular pressure; ED$_{Na}$, early distal NaCl concentration; V$_{ur}$, urine flow rate; Na$_{excr}$, sodium excretion rate; PRC, plasma renin concentration; P$_{Na}$, plasma sodium concentration; P$_{K}$, plasma potassium concentration; P$_{glucose}$, plasma glucose concentration.

Table 2. The clearance series b

<table>
<thead>
<tr>
<th></th>
<th>Group I, Mannitol</th>
<th>Group II, Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>273.3±3.6</td>
<td>1,421±0.03</td>
</tr>
<tr>
<td>KW, g</td>
<td>124±3</td>
<td>116±4</td>
</tr>
<tr>
<td>V$_{ur}$ μl/min·g KW$^{-1}$</td>
<td>12.3±3.0</td>
<td>68.2±9.2</td>
</tr>
<tr>
<td>Na$_{excr}$, nmol·min$^{-1}$·g KW$^{-1}$</td>
<td>2,621±632</td>
<td>5,043±1,219</td>
</tr>
<tr>
<td>K$_{excr}$, nmol·min$^{-1}$·g KW$^{-1}$</td>
<td>5.14±0.34</td>
<td>4.62±0.38</td>
</tr>
<tr>
<td>GFR, μl/min·g KW$^{-1}$</td>
<td>884±56</td>
<td>749±41</td>
</tr>
<tr>
<td>C$_{in}$ μl/min·g KW$^{-1}$</td>
<td>255±23</td>
<td>280±31</td>
</tr>
<tr>
<td>calc APR, μl/min·g KW$^{-1}$</td>
<td>629±42</td>
<td>493±24</td>
</tr>
<tr>
<td>PRC, ×10$^{-8}$ GU</td>
<td>36.2±7.4</td>
<td>53.5±12.6</td>
</tr>
<tr>
<td>Hct, %</td>
<td>44.9±0.5</td>
<td>41.1±0.7</td>
</tr>
<tr>
<td>P$_{glucose}$, mM</td>
<td>6.7±0.4</td>
<td>10.8±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ (paired t-test); KW, kidney weight; K$_{excr}$, potassium excretion; RBF, renal blood flow; GFR, glomerular filtration rate; calc APR, calculated absolute proximal reabsorption rate; Hct, hematocrit; C$_{in}$, lithium clearance.
The series b includes whole kidney data given in Table 2. RBF decreased gradually after mannitol infusion and remained depressed 0.5 ml·min⁻¹·g KW⁻¹ below control value during the 20-min infusion. Because of the decrease in Hct from ~45 to 41%, RPF remained unchanged (from 2.8 to 2.7 ml·min⁻¹·g KW⁻¹).

GFR decreased after mannitol to 85% of the control value, whereas lithium clearance did not increase significantly. From these data, APR after mannitol was calculated to 78% of the control value. In this series, urine flow, sodium excretion, and PRC also increased. Potassium excretion rate remained unchanged (Table 2) as did plasma potassium concentration (Table 1).

During the glucose-water loading (group II), RBF remained unchanged, whereas RPF increased from 3.30 to 3.76 ml·min⁻¹·g KW⁻¹ (P < 0.001) due to the decrease in Hct from ~46 to 41%. Neither urine flow, sodium excretion, GFR, nor lithium clearance changed. Therefore, APR also remained unchanged after glucose as did PRC. Potassium excretion rate decreased significantly, but the decrease in plasma potassium concentration was insignificant (Table 1). The change in plasma glucose was similar to the one in series a (Table 2).

**DISCUSSION**

In the micropuncture series a and c, the rats were fed a diet with a sodium content of 87 mM/kg for comparison with previous studies; whereas in the clearance series b, the diet contained 200 mM sodium to avoid distal lithium reabsorption, which is known to occur at dietary sodium content <50–75 mM (27). This difference in intake resulted in higher sodium excretion rates and urine flows in series b than in series a and c, but these differences have no influence on our conclusions. The higher KW in the mannitol group I in series b compared with the control glucose group II is due to the intrarenal trapping of TF in proximal tubules by mannitol, as seen from the wide open proximal lumina after interruption of blood supply as opposed to the occluded lumina, emptied by reabsorption and drained by the renal vein seen in nondiuretic rats (unpublished observations). This may account for the differences in Vₜw renal clearances of [⁵¹Cr]EDTA and lithium, and RBF and RPF (given per gram of KW) in the control periods in the two groups. But this systematic bias does not influence the observed changes induced by either mannitol or glucose.

The demonstration that EDNaCl decreases during osmotic mannitol diuresis confirms the first study by Ullrich et al. (29) and the later study by Churchill et al. (4), but the results disagree with results from Seely and Dirks (23) in dogs and from a study in inactin-anesthetized Munich-Wistar rats (3). We believe that the most likely explanation for our discrepancy with the last two studies (3, 23) is a difference in the micropuncture technique. They collected TF at a rather brisk rate and without controlling tubular pressure, whereas we recorded the distal conductance continuously by slow fluid aspiration and with simultaneous measurement of the Pₚ₀ₓᵦᵦ (26).

In the present study, PRC increased after the infusion of hypertonic mannitol. In view of the concomitant fall in EDNaCl and the constant arterial pressure, the most likely explanation for the increase in PRC is activation of macula densa-mediated renin release, as shown both in vivo (12) and in vitro (24). The possible importance of the macula densa-mediated renin release in the control of PRC was further emphasized by the first in vivo demonstration of an inverse relationship between EDNaCl and PRC within the physiological...
range of variation in NaCl concentration close to the macula densa. Churchill et al. (5) were unable to find any relationship between EDNaCl and renin secretion rate (or plasma renin) in Na-loaded, control, and Na-deprived rats. Instead, a clear inverse relation between ED-Na load and renin secretion was apparent in their experiments. Reasons for this apparent discrepancy could be different protocols or differences in techniques with TF collection, suspected from their higher values of EDNaCl (62 vs. the present 43 mM). Also, they did not obtain a similar wide range of ED NaCl as obtained in the present study (22–56 mM), because they found no significant difference in EDNaCl between control and Na-deprived rats (69 mM).

In a later study, Churchill et al. (4) observed that plasma renin activity and secretion rate decreased both after saline and mannitol infusion no matter whether EDNaCl increased or decreased. These results
are difficult to compare with ours because they infused 10% mannitol to give volume expansion equal to ~10% of body weight and a marked decrease in $P_{Na}$ concentration from 140 to 119 mM. A most likely explanation for their failure to find an increase in plasma renin after mannitol infusion could therefore be the plasma dilution and that renin release in their experiments was dominated by extrarenal factors such as e.g., decreased sympathetic activity due to the massive volume expansion.

We observed that RBF decreased gradually ($P < 0.01$) during mannitol infusion, whereas RPF, as estimated from RBF and Hct, remained unchanged. By contrast, Blantz (2) reported that RPF in rats increased, whereas RBF remained unchanged after a similar osmotic load. In both studies, the changes were moderate (~10 and 15%, respectively), and again, differences in methodology might give rise to different results. Blantz calculated RBF from the Hct and RPF, and RPF from the clearance of [14C]inulin and inulin extraction ratio as opposed to the present direct recording of RBF.

From our clearance measurements, we conclude that APR decreased as one would expect from an osmotic diuretic. GFR was decreased by ~14%, which is consistent with previous findings (16).

In view of the marked increase in $P_{prox}$ (12–13 mmHg), the fall in GFR was remarkably moderate. If unopposed, such an increase in $P_{prox}$ would cause a fall in GFR to almost zero. This suggests that the filtration pressure [glomerular capillary hydrostatic pressure – mean glomerular oncotic pressure ($P_{gc} - \pi$)] must have increased almost parallel with the tubular pressure. Hct only decreased by ~10%, and therefore the oncotic pressure could hardly have decreased the necessary 10–11 mmHg to explain the change. In his experiments, Blantz (2) estimated the decrease in afferent arteriolar oncotic pressure to be ~6 mmHg after mannitol infusion, which caused a similar 11% reduction in Hct (from 55 to 49%). We must therefore conclude that $P_{gc}$ had increased considerably. Three factors are likely to contribute to the increase in $P_{gc}$. First, the fall in $ED_{NaCl}$ is likely to deactivate the TGF mechanism thereby leading to afferent vasodilation. Second, macula densa-mediated renin release may lead to vasoconstriction of the efferent arteriole. Third, the swelling of the kidney that occurs with osmotic diuresis leads to an increased renal interstitial pressure, which, in turn, compresses the peritubular capillaries thereby adding to the increase in postglomerular resistance. Because of the decrease in overall RBF, we conclude that under the present experimental conditions, the increase in postglomerular resistance overrides any concomitant decrease in preglomerular resistance. This interpretation is consistent with the results from inhibition of proximal reabsorption rate with acetazolamide, which also caused a reduction in RBF and GFR and a marked transient increase in tubular pressure (18).

When a similar water load was given without unabsorbable solute, there was no change in RBF, whereas RPF increased from 3.3 to 3.8 ml·min⁻¹·g KW⁻¹ due to the fall in Hct. Also, GFR, lithium clearance, and estimated proximal reabsorption rate remained unchanged. It may therefore seem surprising that $ED_{NaCl}$ increased slightly (3.6 mM). However, due to the variance of the clearance measurements, we cannot exclude that superficial nephron Henle loop flow might have increased slightly. It may also seem surprising that urine flow did not increase after the water load, but this is consistent with the long-known experience that water diuresis cannot become manifest in anesthetized and laparotomized rats unless they are anesthetized with either inactin or ethyl alcohol (28). The decrease in potassium excretion is probably the expected consequence of insulin secretion in response to the elevated plasma glucose concentration.

We conclude from this set of control experiments that the fall in $ED_{NaCl}$ after mannitol was not due to the volume expansion.
The present isotonic sodium loading given over 30 min corresponds to about one-third of the 24-h intake on a normal rat pellet diet and a total extracellular volume expansion by 0.7% of body weight. It is therefore considered to be modest. It caused a small (6.4 mM) increase in EDNaCl and sodium excretion rate almost doubled. Renin release only showed a minor insignificant decrease. This is probably due to a hyperbolic relationship between changes in distal sodium and renin secretion, similar to the relationship between changes in sodium excretion and changes in plasma renin (Fig. 5). In contrast to osmotic diuresis, there was a significant inverse relationship between the change in sodium excretion and the change in PRC (Fig. 5), which emphasizes the importance of the sodium-retaining effect of the renin-angiotensin system under these “physiological” conditions.

The estimated 22% reduction in APR after osmotic diuresis is in accordance with the long-accepted view of the site and mechanism of action of this type of diuretic (6). However, the total fall in fluid reabsorption in these nephron segments was mirrored by a similar fall in filtered load as seen from the unchanged lithium clearance. Because the sodium concentration at the end of the proximal tubule is decreased during mannitol diuresis, our data suggest that sodium delivery into the tDLH was reduced after mannitol administration. This questions the assumption that the increase in sodium excretion rate during osmotic diuresis is caused mainly by a reduction in sodium reabsorption in the proximal tubule and thick ascending limb of the loop. Furthermore, the fact that we could not detect any significant relationship between the changes in EDNaCl and sodium excretion rate neither during osmotic diuresis nor water or sodium loading supports the view that the major site of regulation of sodium excretion is the cortical- and medullary-collecting ducts even during osmotic diuresis. The absence of a change in potassium excretion during osmotic diuresis is therefore compatible with this interpretation, because an increased distal delivery of sodium should have increased the potassium excretion. The mechanism responsible for the inhibition of collecting duct sodium reabsorption during mannitol diuresis remains unsettled, because it cannot be explained by the increased activity of the renin-angiotensin system.

In summary, we conclude that osmotic diuresis is associated with a decrease in EDNaCl, an increase in renin release, and probably afferent vasoconstriction. These results are compatible with the assumed influence of macula densa on renin release and afferent vascular tone. In addition, our data show that PRC varied inversely with EDNaCl over a wide range, suggesting that macula densa-mediated renin release may be a major determinant of PRC. The results suggest that osmotic natriuresis depends on inhibition of sodium and water reabsorption in the segments of the nephron that are located downstream from the distal convoluted tubule.

**Perspectives**

The aim of the present experimental study was to provide further insight into the physiological mechanisms involved in acute osmotic diuresis with the use of mannitol in an amount that results in a concentration of 35–45 mM in the glomerular ultrafiltrate (16). The new information provided was that EDNaCl decreased and was inversely related to PRC over the physiological range of variation. There was no significant relationship between EDNaCl and sodium excretion either during osmotic diuresis, hypotonic glucose, or sodium loading. A similar high concentration of osmoles as obtained in the present study is seen in certain clinical conditions, such as hyperosmolar nonketotic diabetic coma, but it is not achieved in the more slowly developing osmotic diuresis that accompanies diabetic mellitus and renal failure. Although the described effect of osmoles in the distal nephron and collecting duct may also be highly relevant in these conditions, the acute changes in renal hemodynamics described in this study may be more relevant for the understanding of acute effects of osmotic loading, such as the changes in kidney function seen after infusion of contrast media (21).

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