Original Article

Segmental reabsorption measured by micropuncture and clearance methods during hypertonic sodium infusion in the rat

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Abstract

Background. We wanted to validate by direct measurements in rat tubules a technique used to calculate segmental volume absorption by each segment of the human nephron.

Methods. Experiments were performed on 17 rats during hypertonic Na infusion prior to and after frusemide administration. Tubular samples were taken from early distal and last proximal sites. The rate of filtration of single nephrons (SNGFR) was calculated by the technique of total collection of tubular fluid using labelled inulin as a marker. Reabsorption was computed by the tubular fluid to plasma (TF/P) inulin concentration ratio.

Results. SNGFR was 50±4 nl/min at the distal (n = 82), 51±3 nl/min at the proximal sampling site (n = 112, P>0.65) during baseline conditions. Percent reabsorptions were 85±1 and 69±2% respectively (P<0.0001). During frusemide these values were 52±6 nl/min and 76±2% at the distal, 49±5 nl/min and 66±2% at the proximal site. In 83 paired proximal collections, fractional (68±1% versus 67±1%, P>0.32), absolute reabsorption (34±2 versus 33±2, P>0.50) and SNGFR (50±2 nl/min versus 50±3 nl/min, P>0.99) were not different between baseline and frusemide. In 25 re-collections from the distal tubule these same values were 83±2% versus 76±2%, and 48±4 nl/min versus 55±6 nl/min respectively. Very similar results were obtained in 55 paired distal-proximal collections during baseline, and 42 such pairs during frusemide.

In the presence of the diuretic the fractional urine excretion was significantly correlated (R=0.83, P<0.0001) with fractional proximal delivery. Na+ resorption by Henle's loop was 22±2% calculated from clearance data and 23±1% of GFR from micropuncture data respectively. They were not significantly different (P>0.70) and were significantly correlated (R=0.57, P<0.02).

Conclusions. These data demonstrate that frusemide does not act proximally and that delivery beyond the proximal tubule approximates urine flow rate during the action of the drug. The values of segmental reabsorption along the nephron computed on clearance measurements are superimposable upon those obtained directly by micropuncture.

Key words: Henle's loop reabsorption; frusemide; micropuncture; Na transport; proximal reabsorption; SNGFR

Introduction

Clearance techniques yielded a large body of information on kidney function, but failed to pinpoint the sites of altered Na and water transfer in different segments of the nephron. This led to the development of micropuncture techniques and direct sampling from the tubules of animals.

We developed a new method to calculate reabsorption by the segments of the human nephron in vivo by clearance techniques only, during conditions of maximal water diuresis [1]. We demonstrated the validity of the method by reproducing in rats the clearance data together with the direct measurements obtained by micropuncture, showing the virtual identity between the data of segmental reabsorption obtained by these two independent and different techniques [2]. Our clearance calculations of segmental tubular reabsorption can be extended to conditions of maximal antidiuresis [3]. In this situation, the loop of Henle (HL) dilutes the tubular fluid, as during water diuresis, generating solute-free water (CH2O-HL) within the tubular lumen. This volume, and that due to distal reabsorption (CH2O-DT) are reclaimed by the distal tubule, when ADH is present, till isotonicity is attained. The water abstraction from collecting ducts (CD) into the hypertonic interstitium (TcH2O) results in urine concentration. When frusemide (F) is injected in this antidiuretic condition, CH2O-HL is not formed, the countercurrent mechanism is blocked so that no water is abstracted by the medullary and papillary interstitium, made isotonic by the diuretic [4]. Only CH2O-DT is generated and reabsorbed [5]. Based on the difference between urine flow rates and TcH2O measured...
before and during F, a suitable set of algebraic equations, detailed in the methods, allows the calculation of all these volumes, mainly CH₂O-HL and fractional delivery of filtrate from the proximal tubule. In essence, CH₂O-HL and TcH₂O are delivered into the urines during F, and can be computed by difference with respect to baseline urine flow.

The method is based on the critical assumption that F does not inhibit proximal reabsorption. Up to the last proximal (LP) sampling site available on the renal surface we could confirm this assumption in micro-puncture experiments [6], in agreement with results from others [7-10].

The data on segmental tubular reabsorption with the method valid during maximal ADH secretion were gathered only in man [3], and there is no study that reproduces them in animals, and validates them by simultaneous direct micropuncture evidence during hypertonic Na infusion.

In the present paper we report data obtained during antidiuresis by hypertonic Na infusion in rats, studied by clearance techniques and by direct sampling of last proximal (LP) and early distal (ED) tubular segments.

Methods

The experiments were performed on 17 Wistar rats, weighing 220-350 g. The animals were anaesthetized with Trapanal (Byk Goulden, Konstanz, Germany) 100 mg/kg, and prepared for micropuncture as already described [2,6]. This preparation includes a urine reinfusion system which is started as soon as both ureters have been cannulated.

Immediately after the cannulation of the jugular vein, we administered 5 ml of a priming solution that contained 250 mmol/l NaCl, followed by a continuous infusion at a rate of 2.2 ml/h. After the urine reinfusion was started, the hypertonic Na infusion was continued. In addition we administered 135 μC/kg of the glomerular marker 14C-inulin (Amersham International, Buckinghamshire, England), which was continuously refed to the animal through the urine reinfusion system. This resulted in stable steady-state plasma concentrations. After an average of 50 min, the control period (B) began, during which 2–3 clearance periods were performed, and 7–16 nephrons were sampled. Three blood samples were taken, one after each clearance period.

The timed urine collections lasted the time necessary to fill a calibrated volume capillary, on average 5 min. When frusemide, 10 mg/kg, was injected. While the urine reinfusion system continued, the hypertonic maintenance was reduced to 1.2 ml/min. After 30 min the experimental period (F) was started: again three clearance periods with timed collection of a predetermined urine volume, and sampling from 4 to 16 nephrons were performed. Therefore, the urine reinfusion system was performing the hypertonic reabsorption, freeing the hyper-natraemia, the maintenance infusion was meant to maintain the maximal ADH output while replacing the insensible losses, those due to sampling, and the leakage through surgical wounds.

The tubules were identified by the injection of two small boluses of a solution containing 10% lissamine green through a very thin tipped pipette (<3 μM OD), and by observing the appearance and disappearance of the dye along the last proximal convolutions and the early distal segment of the same nephron. The proximal and distal transit time were recorded. The nephrons were mapped and sampling took place, when feasible, from the last proximal (LP) and early distal (ED) loops accessible to micropuncture on the renal surface. During baseline (B) we collected from the isolated distal tubules (which were recollected during F), from the isolated proximal tubules (which were recollected during F), and from couples of ED and LP tubules. These were collected only during B, and the ED sampling always preceded the LP collection from the same nephron. During F we sampled again from ED and LP sites of the same nephrons, although different from those used during B. In addition, during F we re-collected the isolated proximal and distal tubules already sampled during B. Finally, a number of isolated nephrons were sampled either at ED or LP, during B or F. These isolated nephrons were either those where the initial collection was lost during sample processing, or tubules that could not be re-collected for technical reasons, mainly persistence of the oil block and leakage from the previous puncture site. In summary, each tubule was always punctured twice, either from the same site, or from different sites (distal and proximal).

Details of the collection technique, measurement of tubular fluid (TF) and plasma (P) inulin, and calculations of nephron filtration rate, fractional and absolute reabsorption were described previously [2,6,11], together with details of clearance measurements, urine Na and osmolality determinations, and calculations of Na excretion and GFR.

In the present paper we shall use the following calculations: SNGFR = nephron filtration rate, in nl/min; FR, fractional reabsorption, calculated as 1−P/TF inulin; PR, percentage reabsorption, calculated as FR × 100; AR, absolute rate of reabsorption in nl/min; CR, collection rate from the sampling pipette, in nl/min; PD, proximal delivery, calculated as 1−FR × 100, expressed as percentage of GFR.

We also calculated with conventional formulae the osmolar clearance (Cosm), the water reabsorbed across collecting ducts (TcH₂O), and urine flow rate during B (V) and during F (VF), and expressed them in absolute values (μl/min) and as %GFR. With different formulae that we developed [1,3] we also calculated the Na transport by the loop of Henle, expressed as the volume of solute-free water generated (CH₂O-HL), and expressed it as %GFR. The theory on which these calculations are based was published by guest previously [1,3]. Briefly, during maximal antidiuresis and hypertonic Na infusion, CH₂O-HL is generated during water diuresis. Along the distal convoluted tubule this volume of solute-free water, and that formed by distal Na reabsorption (CH₂O-DT), are reabsorbed, and the urine reaches isotonicity with respect to plasma. While travelling along the collecting ducts (CD) across the hypertonic interstitium, more water is abstracted (TcH₂O) and the urine becomes hypertonic. Hence, the urine flow rate (V) during baseline antidiuresis (B) is given by the delivery from the end of the proximal tubule (PD, proximal delivery) minus CH₂O-HL (the free water formed by the loop), CH₂O-DT (the free water formed by the distal tubule, equivalent to distal Na absorption in excess of K secretion), and TcH₂O:

\[
V = PD - (CH₂O-HL + CH₂O-DT + TcH₂O) \quad (1)
\]

During F we assume that PD is unchanged [2,6], CH₂O-HL is not generated [5], TcH₂O is not reabsorbed because of the disappearance of the medullary and papillary gradient across CD [4], while CH₂O-DT is formed and reabsorbed [12]. Hence,

\[
V_F = PD - CH₂O-DT \quad (2)
\]
Work' statistical program applied to a Macintosh LC II expansion during F as the haematocrit fell from 47 to 45 ± 1%, as '%GFR', computed from the averages obtained in clearance by the TAL of HL, are expressed in ul/m/100 ul GFR ('% of GFR'); UNa and PNa, urinary and plasma sodium concentration.

The results obtained by clearance techniques on the 17 animals studied. In each rat there was a separate average calculating their means from the average value obtained in each of these same figures were 62 and 135 respectively. SNGFR is the rate of filtration of individual nephrons in nl/min. PR is the percentage reabsorption. The PD is the percentage of filtrate delivered beyond the TAL of HL can also be computed from micropuncture data, and express them as ml/min/100 ml GFR. CH2O-DT can be obtained by mixed micropuncture and clearance data, and By subtracting 1 from 2 we obtain:

\[
\text{CH}_2\text{O}-\text{HL} = V_f - V - \text{TcH}_2\text{O} 
\]

Therefore, with these formulae, we can calculate CH2O-HL by clearance data, and express them as ml/min/100 ml GFR.

The results were analysed statistically, means and standard errors of the mean were computed, together with statistical differences between means by paired or unpaired t tests. Regressions and correlations between variables were also calculated. All statistical work was performed by the 'Stat .Work' statistical program applied to a Macintosh LC II personal computer.

### Results

The results obtained by clearance techniques on the 17 rats are reported in Table 1. GFR did not change during F with respect to B, while urine flow rate and Na excretion rose sixfold. The animals were in maximal antidiuresis during sustained osmotic load by hypertonic Na infusion, as indicated by the baseline urine and plasma osmolality and by the presence of TcH2O of 2.6 ± 0.3 ml/min/100 ml GFR. During F the urine became isotonic to plasma, while PNa was unchanged. Frusemide exerted its known effects, which are qualitatively quite similar to those we observed in normal humans [3]. There was a slight additional volume expansion during F as the haematocrit fell from 47 ± 1 to 45 ± 1%, P < 0.004. The clearance data are reported as '%GFR', computed from the averages obtained in each rat.

Table 2 reports all data obtained by micropuncture techniques, independent of whether they were obtained by re-collections, single tubules, or distal–proximal collections. They were obtained by computing the means from the average numbers calculated in each animal. These values are trivially different from those obtained by the overall tubular samples, irrespective of single rats, reported in the summary.

The SNGFR was not different when measured at the ED or LP site, neither was it different during B with respect to F. Both absolute and fractional reabsorptions were unchanged at the end of the accessible portion of the proximal tubule, indicating that the impressive diuretic effect of frusemide must have occurred beyond it. During B 85% of GFR had been reabsorbed at the ED, 69% at the LP. During F, these figures were 76 and 67% respectively. While there was no statistical significance at the LP, the difference was significant at the ED. However, the ED-LP difference was significantly higher during B (16 ± 1%) than during F (11 ± 2%, P < 0.04). The micropuncture-derived values are very similar to those obtained by clearance.

### Table 1. Clearance data in 17 animals

The Table reports the clearance data (± the standard errors of the means) obtained in 17 rats during B and after the administration of F. The P values refer to the paired statistical comparisons between B and F for the measurements reported (P B vs F). HS means highly significant (P < 0.0001). NS = non-significant (P>0.05).

<table>
<thead>
<tr>
<th>μ/min GFR</th>
<th>%GFR V</th>
<th>TcH2O</th>
<th>CH2O-HL</th>
<th>mmol/l UNa</th>
<th>PNa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1180 ± 86</td>
<td>5 ± 1</td>
<td>2.58 ± 0.29</td>
<td>155 ± 15</td>
<td>163 ± 1</td>
</tr>
<tr>
<td>P B vs F</td>
<td>NS</td>
<td>HS</td>
<td>HS</td>
<td>23.0 ± 1.7</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Frusenimde</td>
<td>1220 ± 86</td>
<td>31 ± 2</td>
<td>-1.46 ± 0.63</td>
<td>154 ± 7</td>
<td>163 ± 3</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate in microlitres per minute (μl/min); V, urine flow rate; TcH2O, free-water abstraction; CH2O-HL, free water clearance by the TAL of HL, are expressed in μl/m/100 μl GFR (% of GFR); UNa and PNa, urinary and plasma sodium concentration (in mEq/l) respectively; UNa V, urine sodium excretion, in microequivalents per minute, Uosm, urine osmolality, in milliosmoles per kg of water; U/Pin is the urine to plasma inulin concentration ratio.

### Table 2. Micropuncture data in 391 tubules of 17 rats

The Table reports all micropuncture data (391 tubular samples) by calculating their means from the average value obtained in each of the 17 animals studied. In each rat there was a separate average value for each of the measurements reported during B (194 tubules) and during F (197 samples) respectively. During baseline, 82 samples were obtained from ED, 112 from LP segments. During frusemide, these same figures were 62 and 135 respectively. SNGFR is the rate of filtration of individual nephrons in nl/min. PR is the percentage reabsorption. The PD is the percentage of filtrate delivered beyond proximal sampling site and is calculated as 100 – PR.

CH2O-HL and CH2O-DT are computed from micropuncture data only and expressed as '%GFR'.

| μ/min/GFR · 100 | LP SNGFR | 51.5 ± 2.9 | NS | 48.8 ± 5.0 |
| PR %PD | 69 ± 2 | NS | 66 ± 2 |
| LP SNGFR | 49.9 ± 4.5 | NS | 51.7 ± 6.0 |
| PR ED | 85 ± 1 | HS | 76 ± 2 |
| CH2O-HL | 21.2 ± 1.3 | 2.9 ± 1.2 |
| CH2O-DT | 

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computation of segmental transport reported in Table 1. In fact micropuncture delivery and clearance reabsorption add to 100% of GFR. The fractional urine flow, loop and distal tubule resorptions, beside matching their paired micropuncture estimates, are also close to the means obtained in man.

Figure 1 reports paired micropuncture data only. They were obtained in 83 LP segments sampled during B, and re-collected during F. Even these paired data demonstrate that F does not change SNGFR, fractional and absolute reabsorption at the end of the accessible portion of the proximal tubule. This figure also reports the data obtained in 25 ED segments sampled during B, and re-collected during F. These paired data demonstrate that F induces a slight albeit significant fall in volume reabsorption at the ED sampling site.

Figure 2 shows additional paired data. They were obtained during B in 55 tubules, sampled twice: the first collection was from the ED site, the second collection was taken immediately afterwards from the LP sampling site of the same nephrons. This procedure was repeated in 42 different nephrons sampled during F. The data confirm those shown previously: SNGFR was not different when measured at ED with respect to LP sites of the same nephrons, both during B and F. There was a significant paired difference in fractional reabsorption between ED and LP sites, both during B and F. The absolute rate of reabsorption at LP was not different, by unpaired t test, during B with respect to F (P > 0.05).

Table 3 reports the data of regression equations and correlations between some of the variables measured.

Table 3. Correlations between micropuncture and clearance measurements in 17 rats

<table>
<thead>
<tr>
<th>Independent variable (X)</th>
<th>Dependent variable (Y)</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNGFR</td>
<td>GFR</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SNGFR-LP</td>
<td>SNGFR-ED</td>
<td>0.35</td>
<td>HS</td>
</tr>
<tr>
<td>LP Delivery</td>
<td>ED Delivery F</td>
<td>0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average delivery</td>
<td>CH₂O-HL</td>
<td>0.55</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Average delivery</td>
<td>CH₂O-DT</td>
<td>0.30</td>
<td>NS</td>
</tr>
<tr>
<td>V/GFR F</td>
<td>LP Delivery F</td>
<td>0.80</td>
<td>HS</td>
</tr>
<tr>
<td>V/GFR F</td>
<td>ED Delivery F</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>V/GFR F</td>
<td>Average delivery</td>
<td>0.82</td>
<td>HS</td>
</tr>
<tr>
<td>V/GFR F</td>
<td>CH₂O-HL</td>
<td>0.75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>V/GFR F</td>
<td>CH₂O-DT</td>
<td>0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Δ-PR B</td>
<td>CH₂O-HL</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Δ-PR B</td>
<td>CH₂O-DT</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Δ-PR F</td>
<td>CH₂O-DT</td>
<td>0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Δ-PR F</td>
<td>CH₂O-DT</td>
<td>0.25</td>
<td>NS</td>
</tr>
<tr>
<td>V b</td>
<td>C Osm b</td>
<td>0.97</td>
<td>HS</td>
</tr>
<tr>
<td>V f</td>
<td>C Osm f</td>
<td>0.93</td>
<td>HS</td>
</tr>
</tbody>
</table>

The Table reports the correlations computed from the average data obtained on each of the 17 rats studied, whose means are reported in Tables 1 and 2.

For each correlation the Table shows the independent variable (X), the dependent variable (Y), the correlation coefficient (R), and its significance (P). The average delivery is the mean of two measurements of delivery, that beyond LP and that beyond ED during F. The symbol delta (Δ) indicates the difference between ED and LP data in the reported measurements. The symbol B and F refer to baseline and frusemide and are reported in lower-case letters in the two correlations at the bottom.
crit was also substantially stable. It averaged 47 and 45% during B and F respectively, values significantly lower than those measured in different animals during isotonic maintenance infusions, of 50 ± 1% (P < 0.01).

The finding that both GFR and SNGFR were stable during the experiment (Table 1). The haematocrit was also substantially stable. It averaged 47 and 45% during B and F respectively, values significantly lower than those measured in different animals during isotonic maintenance infusions, of 50 ± 1% (P < 0.01).

Discussion

The present study validates during antidiuresis a method which had already been validated in maximal water diuresis [2]. The original method was tested on humans and found to give reproducible calculations of volume and Na transfer by different segments of the nephron, both during water diuresis [1] and antidiuresis [3]. The present study reproduces in animals the method devised for antidiuretic conditions, and the same rates of hypertonic Na infusion were adapted to rats to closely mimic the human study, since the aim of the experiment was to offer to the scientific community a reliable method applicable to investigations on clinical physiology. The constancy of the extracellular fluid volume and Na concentration was assured by the urine reinfusion technique, already validated in maximal isotonic maintenance infusions, of 50±1% (P>0.01).

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Assumption (b) is validated by an undisputed body of evidence [5], and this also applies to the last assumption [4]. The third assumption is based on the findings that Na transport is unchanged along the distal tubule by F [19], and that it is not flow dependent during microperfusion experiments [20].

However, there was a difference in volume absorption between proximal and distal sampling sites in the present experiments, and this difference was significantly reduced by the diuretic. F must then have decreased volume transport along at least one of the segments between the two sampling sites, either the pars recta, or the thin descending and ascending limbs, or the early distal tubule, neglecting the TAL, which is impermeable to water.

It is possible that F may act along the pars recta. For instance, its phosphaturic effect [21], though relevant, was substantially stable. It averaged 47 and 45% during B and F respectively, values significantly lower than those measured in different animals during isotonic maintenance infusions, of 50 ± 1% (P < 0.01). The finding that both GFR and SNGFR were unchanged from B to F demonstrates the constancy of body fluid compartments. Their ratio was 24.20 ± 1.32 in B and 25.53 ± 1.63 in F, P>0.53. This gives the thousands of glomeruli per kidney, in agreement with counts of others [13]. The slope of the regression between GFR and SNGFR (22.64, Table 3) yields a similar number of glomeruli.

The technique investigated is based on the assumptions that (a) F does not act proximally, (b) free water generation in Henle’s loop occurs along the frusemid-sensitive thick ascending limb (TAL), (c) distal tubule free water generation is not affected by F, and (d) TCH2O is not absorbed by collecting ducts in the presence of F.

Concerning the first assumption, our present data, together with those of previous papers [2,6] and with those of others [7–10] demonstrate that in the face of stable GFR and SNGFR, fractional and absolute proximal reabsorptions remain unchanged. The dose of F used was 10 times that necessary to elicit maximal natriuresis. Most studies claiming a proximal effect of F, as recently reviewed by us [2,6], were complicated either by unstable renal haemodynamics with the diuretic, or by overexpansion produced in the attempt to compensate the urinary losses induced by the drug. Others reported an inhibitory effect only with high F doses [14], although this could not be seen even with 100 mg/kg in a single study [15]. We cannot dismiss our own 151 paired last proximal samples before and after F in the present and two previous studies [2,6]: the pooled data show that SNGFR was 42.5 ± 1.5 before and 41.5 ± 1.4 nl/m after F (P > 0.48), and that percent reabsorption was 71.72 ± 1.23% before and 71.72 ± 1.16% after F (P = 1.00). Thus we believe we obtained conclusive evidence that F has no proximal effect on volume transport up to the last accessible convolution of the PT. Carbonic anhydrase inhibition may not occur in vivo because of negligible filtration due to protein binding of F [16], which is secreted in the accessible tubular segments [17]. However, F-induced bicarbonaturia was not confirmed in recent studies, where bicarbonate absorption was shown to be increased along Henle’s loop by F via a carbohydrase-dependent system [18].
Segmental tubular reabsorption

A final point to be clarified in reference to the discussion on the methods used, is represented by the measurements of SNGFR from the distal and proximal sampling sites. The present study, in agreement with the previous ones [2, 6], discloses no difference in paired and unpaired values of ED versus LP filtrations. Although this is important, as it allows meaningful comparisons of data, it raises the question as to whether our technique is adequate to disclose differences predicted by the tubuloglomerular feedback mechanism. While it is not surprising for SNGFR to be the same at both sites during F, since the drug shuts off the macula densa [9], the lack of difference during B is more difficult to account for. However, the present experiments were performed during hypertonic expansion, which may have fully vasodilated the kidney. The system undergoes a resetting in conditions of maximal vasodilatation, such as those occurring near the lower limit of autoregulation or during volume expansion [30].

Thus, we believe that the assumptions on which our method is based are reasonable and are supported by the direct evidence obtained in our experiments.

The data gathered previously during water diuresis are then confirmed with an entirely different experimental protocol suitable for maximal antidiuresis. This was obtained by hypertonic Na infusion, which causes a diuretic condition in the presence of maximal ADH secretion, maximal Henle’s loop transport, and interstitial hypertonicity. The first goal of our experiments, as already discussed, was to verify whether F interferes with proximal reabsorption during hypertonic infusion.

The second goal concerns HL transport. When measured, by clearance techniques, as the equivalent volume of free water formed, CH₂O-HL was 23.0 ± 1.7 ml/min/100 ml GFR, a value quite close to that measured in humans during antidiuresis [3] and in humans [1] and rats [2] during water diuresis. In the previous studies we demonstrated by regression equations that CH₂O-HL was 69% of proximal delivery (PD). In this experiment we measured PD and were then able to compute CH₂O-HL with a clearance-independent method. The value we obtain, reported in Table 2, is 21.2 ± 1.3 ml/min/100 ml GFR, in full agreement with that computed from clearance data on the same animals. Furthermore, these two estimates of CH₂O-HL are significantly correlated (R = 0.57, P < 0.02).

We measured volume transport, which in the PT is equivalent to Na transport. In HL it would require measuring the Na concentration in the tubular fluid. However, our formulae allow calculation of the volumes of solute-free water generated, which can be considered equivalent to the salt reabsorbed without solvent. The assumption that F completely blocks TAL transport is validated by all experiments in vivo [15] and in vitro [5] and does not require direct confirmatory measurements.

Thus our original method yields consistent estimates of HL transport in humans and animals, which agree with more direct measurements obtained by micro-
puncture. A similar conclusion can be reached for distal Na reabsorption, expressed as CH₂O-DT, the solute-free water generated by the DT. It can be estimated in the present experiments as the difference between PD (a micropuncture measurement) and Vf (the urine flow rate per 100 ml of GFR during F). The value reported in Table 2 is 2.9 ± 1.2 ml/min/100 ml GFR, not significantly different (P > 0.60) from that measured during water diuresis [2]. This estimate is valid under the assumption that F does not affect distal Na transport, as demonstrated by others [19]. Its validity depends on the additional assumption that any water abstraction by thin descending limbs, computed as CH₂O-HL by our method, does not induce a flow-dependent change in distal reabsorption. The lack of flow dependency was shown by perfusion of HL at different rates [20].

The final point was to confirm the relationship between PD and urine flow rate during F. This was shown to be virtually identical and significantly correlated during water diuresis [2], still correlated and with a slight difference during antidiuresis [6]. During hypertonic saline infusion, PD (computed as 100—PR, Table 2) averages 33.6 ± 2.1%, Vf 30.7 ± 2.1%, P < 0.03. They were significantly correlated (R = 0.83, P < 0.0001, Table 3 and Figure 3). In fact PD was slightly in excess of distal delivery, this difference representing, as discussed previously, an estimate of CH₂O-DT.

In conclusion we believe that during F the same volume inflow (as compared to baseline conditions) to the HL will result in a larger Na delivery to the DT, since the Na concentration in the inflowing fluid rises to approximate the plasma value. This abolishes the osmotic gradient between peritubular fluid and lumen, and no further solvent flow across the epithelium will take place in the absence of ADH, as in a previous study where PD and Vf were equal [2]. In the presence of ADH, as in another previous study [6] and the present one, the distal Na reabsorption generates hypotonicity of the tubular fluid; even this slight osmotic gradient will allow the absorption of the solute-free water generated in the lumen, osmotically equivalent to Na reabsorption, that we call CH₂O-DT.

Therefore the present study confirms the validity of our original method of calculating segmental Na reabsorption in vivo by clearance techniques alone [1,3]. We do not intend to transfer the numbers generated from one species to another, but only test the internal consistency of the pathophysiological theory that underlies our calculations of segmental tubular reabsorption. This requires that direct measurements and indirect calculations agree within the experimental error, and that the individual averages calculated in each animal fit together in significant correlations and regressions. Since this was found to be true in the present and the previous study during water diuresis [2], we conclude that volume reabsorption, and the equivalent salt transported by each segment of the human nephron can be estimated in vivo with reproducible accuracy, based on assumptions which were repeatedly verified on experimental animals, provided that the rates of infusions were capable of maintaining a constant degree of volume expansion during the baseline conditions and the subsequent injection of F. For human studies the maximal water diuresis technique [1] is easier to perform and more suitable. The hypertonic infusion technique [3] could represent a useful adjunct to further evaluate derangements of renal function involving the countercurrent system.

References


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