Effect of Dietary Sodium Intake on the Intrarenal Distribution of Nephron Glomerular Filtration Rates in the Rat

By Jaime B. Coelho

ABSTRACT

Redistribution of single nephron glomerular filtration rate (SNGFR) between superficial (S) and juxtamedullary (JM) nephrons is thought to participate in the renal adaptation to different levels of dietary sodium intake. This possibility was examined using the Hanssen technique which measures SNGFR as the $^{14}$C-ferrocyanide content of microdissected nephrons factored by the mean plasma concentration following a 12-second infusion. Rats from the same litter were placed on a low-sodium (0.8 mEq/day) or a high-sodium (9 mEq/day) diet. The distribution of SNGFR was measured as the $S/JM$ (mean of $S$ nephrons/mean of $JM$ nephrons) SNGFR ratio. In spite of an eightfold change in sodium excretion, there was no significant change in the $S/JM$ SNGFR ratio (low sodium - high sodium: $-0.0033 \pm 0.048$ [SE], $P > 0.9$).

Seven rats that were not littermates were maintained on a standard diet but drank saline (13 mEq sodium/day); carotid and ureteral catheterization and the duration of the diet (3-14 weeks) had no effect on the $S/JM$ SNGFR ratio ($P>0.1$), which was similar to that of nine rats maintained on a standard diet with water for drinking (3 mEq sodium/day). The value of the $S/JM$ SNGFR ratios were 0.77 and 0.83, respectively. These variations in dietary sodium intake appeared to have no detectable effect on the intrarenal SNGFR distribution.

KEY WORDS Hanssen technique juxtamedullary nephrons salt balance structural-functional relationships nephron heterogeneity renal circulation renal hemodynamics ferrocyanide clearance

Nephron heterogeneity has been regarded as a potential source of renal functional flexibility (1) that could influence the regulation of sodium balance (2). Horster and Thurau (3) found that a chronic increase in dietary sodium intake gave rise to a redistribution of single nephron glomerular filtration rate (SNGFR) toward the superficial (S) nephrons. Particularly noticeable in their experiments was the marked (71.5%) drop in juxtamedullary (JM) SNGFR that attended the change from a low- to a high-sodium intake. Therefore, these authors have postulated that intrarenal shifts in the sodium load offered to tubules having different reabsorptive capacities may constitute an important mechanism in the chronic regulation of sodium balance by the kidney (3).

Subsequent investigations of this problem have yielded less dramatic results. In preliminary experiments from this laboratory (4), using the Hanssen technique for the measurement of SNGFR distribution (5), a moderate redistribution toward S nephrons was observed in only two of five rats fed a high-sodium diet. de Rouffignac and Bonvalet (6) found that a high sodium intake resulted in a moderate redistribution toward S nephrons but did not confirm the marked fall in JM SNGFR observed by Horster and Thurau (3). However, because de Rouffignac and Bonvalet (6) superimposed an acute hypertonic sodium load, the results are not strictly comparable. Recently, Baines (7) has suggested that redistribution may have a structural basis, but he found that the redistribution was not required for the natriuresis of chronic high-salt intake.
In the present study, the effects of purely chronic changes in dietary sodium intake on the intrarenal distribution of SNGFR were studied using a recently validated modification of the Hansen technique (8). In an effort to minimize the underlying variation inherent in comparisons between rats, littermates were used for parts of the study. The results do not support the concept that a redistribution of SNGFR participates in the chronic regulation of sodium balance.

**Methods**

This study was performed on 24 male albino rats of the Sherman, Wistar–derived (9) strain. Eight rats from three litters were fed a sodium-deficient diet. Five rats drank 0.1% NaCl and 3 rats drank 1.0% NaCl for at least 3 weeks; the approximate daily sodium intake of these groups was 0.8 and 9 mEq/day, respectively. Two of the rats on low-sodium intake were given distilled water to drink during the last 5 days preceding the study to further reduce their sodium intake. The remaining 16 rats were fed a standard diet of rat pellets containing 0.4 g sodium/100 ml; 9 of these rats drank tap water and 7 drank 0.9% NaCl. The approximate sodium intake of these two groups was 3 and 13 mEq/day, respectively. In this latter group the duration of the high-sodium intake varied between 3 and 14 weeks.

Food was withdrawn the night before the study, but saline or water were allowed ad libitum. The rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), and anesthesia was maintained with additional amounts of the drug (5 mg/kg, ip) during the study as required. With this regimen, the level of anesthesia was not deep as judged by the generally positive corneal reflex. All rats underwent tracheal intubation and left jugular vein, femoral artery, and bladder catheterization. Usually, the left carotid artery was catheterized for arterial blood pressure monitoring. However, since Horster and Thurau (3) avoided carotid catheterization, a similar procedure was followed in 3 rats by substituting the contralateral femoral artery for the carotid artery. The right ureter was catheterized in the subcutaneous space. The right kidney was used for microdissection of nephrons and summated SNGFR and the left kidney was used for glomerular counting as previously described (8). The level of extraluminal radioactivity in proximal tubules was estimated in each study by the radioactivity in samples of distal convolutions of known length. It had been observed (8) that the nonfiltered radioactivity per unit tubular length is similar in the distal convolutions and the cortical segments of the proximal tubules, i.e., the initial three-fourths of their total length. The extraluminal radioactivity in the terminal segment was directly measured in some studies, but in others a true value could not be obtained owing to the proximity of the filtered ferrocyanide front. However, the true level was approximately 50% of the radioactivity in distal convolutions, representing only 12.5% of the total extraluminal radioactivity in the complete proximal tubule. Therefore, in later studies its direct measurement was deemed unnecessary, and a value of 50% of the radioactivity in distal convolutions was taken. Distal convolutions from the outer and inner cortex were sampled separately. Because the radioactivity was similar in both regions of a given kidney, regardless of the level of sodium intake, an average was taken and applied to all the nephrons in that kidney. In each study 10–12 nephrons were obtained from each of three cortical layers: superficial, intermediate, and juxamedullary. The criteria for nephron selection, the technique for glomerular counting, the calculations of SNGFR and summated SNGFR (Σ SNGFR), and the radiochemical methods have been described elsewhere.
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(8). Sodium concentration was measured by flame photometry. Statistical analysis was performed according to Snedecor (10), and the mean ± se was used throughout.

Results

The extraluminal, nonfiltered radioactivity in complete proximal tubules (glomerulus-to-thin limb), calculated on the basis of the mean radioactivity in distal convolutions (see Methods), amounted to 21.5 ± 0.7% (se) of the total radioactivity in superficial proximal tubules with glomeruli and 19.1 ± 0.7% in juxtamedullary proximal tubules with glomeruli (P < 0.025). The level of sodium intake had no effect on these percents. The glomerular radioactivity was assumed to represent filtrate in Bowman's space. Undoubtedly, however, a fraction which could not be quantified corresponded to plasma ferrocyanide within the glomerular capillaries. The error introduced by this uncertainty was small, because the total glomerular radioactivity represented only about 9-10% of the total proximal radioactivity. Moreover, this factor did not affect the S/JM~ SNGFR ratio, because, presumably, the nonfiltered glomerular radioactivity accounted for the same fraction of the total in both glomerular populations.

Nonlittermate Rats.—This group included 16 rats on a standard pellet diet. Seven of these rats were changed from water to 0.9% NaCl at different times prior to the study, resulting in an abrupt increase in sodium intake from 3 to 13 mEq. Table 1 shows the individual data for these 7 rats. The sodium ferrocyanide concentration of the infusate was 10% in the first 2 rats and 8% in the other 5 rats.

The effects of left carotid or right ureter catheterization and the duration of the high-sodium diet on the SNGFR distribution were tested by Student's t-test for independent samples (10). The t values were 0.69, 0.99, and 2.16, respectively, with five degrees of freedom (P > 0.10). The individual data for water-drinking rats on a standard pellet diet have been reported elsewhere (8). Only the mean values are shown in Table 1 for comparison with the saline-drinking rats. There were no significant differences in inulin clearance, SNGFR, and $S/JM$ SNGFR ratio between these two groups.

Littermate Rats.—These rats were studied to minimize the rat-to-rat variation, since only one physiological state could be evaluated in any given rat. The sodium ferrocyanide concentration of the infusate ranged between 7% and 8% in this group. The fall in arterial blood pressure associated with this infusion was very small in five of the eight studies (0-4 mm Hg) and did not exceed 20 mm Hg in the other three. In no instance was the final arterial blood pressure lower than 100 mm Hg (Table 2). Rats in this group were compared only to their own littermates, and, because more than one comparison was possible, pairing was done on the basis of the shortest time between the respective studies. Thus, rat A-1 was paired with A-2, rat A-3 was paired with A-4, and rat B-1 was paired with B-2. The mean of the differences between $S/JM$

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrarenal SNGFR Distribution in Nonlittermate Rats: Effect of Carotid and Ureteral Catheterization and Duration of High-Sodium Diet</td>
</tr>
<tr>
<td>CAC</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
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<tr>
<td>Yes</td>
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<td>No</td>
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<tr>
<td>No</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>Mean ± se (n = 6)</td>
</tr>
<tr>
<td>Standard Diet (5 meq/day)</td>
</tr>
</tbody>
</table>

CAC = carotid artery catheterisation. *This rat was not included in the means to preserve the homogeneity of the group. †Individual data for this group have been reported elsewhere (8). The lightest rat was not included in the means to facilitate comparison with the high-salt group.

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TABLE 2
Effect of Daily Sodium Intake on the Intrarenal SNGFR Distribution in Littermate Rats

<table>
<thead>
<tr>
<th>Rat*</th>
<th>Body weight (g)</th>
<th>Kidney weight (g)</th>
<th>Arterial blood pressure (mm Hg)</th>
<th>Urine flow rate (ml/min)</th>
<th>Sodium excretion rate (µEq/min)</th>
<th>Inulin clearance (ml/min)</th>
<th>SNGFR (nliters/min)</th>
<th>S/JM</th>
<th>S/JM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>124→124</td>
<td>2.6</td>
<td>0.14</td>
<td>1.85</td>
<td>22.5</td>
<td>27.5</td>
<td>30.9</td>
</tr>
<tr>
<td>A-1</td>
<td>173</td>
<td>0.647</td>
<td>114→104</td>
<td>4.4</td>
<td>0.26</td>
<td>2.23</td>
<td>26.9</td>
<td>26.7</td>
<td>34.2</td>
</tr>
<tr>
<td>A-2</td>
<td>200</td>
<td>0.879</td>
<td>125→125</td>
<td>7.3</td>
<td>1.83</td>
<td>2.18</td>
<td>25.4</td>
<td>27.1</td>
<td>31.8</td>
</tr>
<tr>
<td>A-3</td>
<td>312</td>
<td>1.08</td>
<td>126→106</td>
<td>6.8</td>
<td>1.45</td>
<td>2.20</td>
<td>30.8</td>
<td>29.6</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>118→100</td>
<td>5.0</td>
<td>0.50†</td>
<td>3.10</td>
<td>38.2</td>
<td>42.5</td>
<td>59.0</td>
</tr>
<tr>
<td>B-1</td>
<td>303</td>
<td>1.02</td>
<td>144→142</td>
<td>5.4</td>
<td>0.50†</td>
<td>3.46</td>
<td>54.9</td>
<td>55.0</td>
<td>81.6</td>
</tr>
<tr>
<td>B-2</td>
<td>395</td>
<td>1.34</td>
<td>148→144</td>
<td>6.2</td>
<td>1.50</td>
<td>4.56</td>
<td>54.4</td>
<td>67.7</td>
<td>88.0</td>
</tr>
<tr>
<td>C-1</td>
<td>283‡</td>
<td>1.05</td>
<td>140→138</td>
<td>4.6</td>
<td>0.11</td>
<td>—</td>
<td>46.2</td>
<td>46.1</td>
<td>63.2</td>
</tr>
</tbody>
</table>

Abbreviations are the same as in Table 1.
* Rat numbers indicate order in which they were studied.
† Arterial blood pressure at the beginning and end of the ferrocyanide infusion.
‡ Bloody urine.
§ Sodium-free diet during the last 5–6 days.

SNGFR low sodium and S/JM SNGFR high sodium was −0.0033 ± 0.048 (P > 0.95). These data, although too few to stand alone, serve to confirm the observations about nonlittermate rats, extending them over a wider range of sodium intake.

In litter A, sodium output during the study averaged 0.20 µEq/min for the two rats on low-sodium intake and 1.64 (or eight times higher) for the two rats on high-sodium intake (Table 2). This finding corresponds reasonably well with an elevenfold ratio of sodium intakes, but extrapolation of the measured sodium output to the whole day shows a substantial lag in excretion relative to intake. It is not known whether this finding represents the effect of anesthesia, the inadequate replacement of surgical losses, or some other factor. In litter B, the sodium concentration in the urine of rats on low-sodium intake was probably erroneously high because of the presence of blood in the urine.

Two additional rats (B-3 and C-1) were kept on a sodium-free diet during the last 5–6 days preceding the study to further expand the range of sodium intake (Table 2). In spite of this diet, the S/JM SNGFR in these two rats fell within the range observed in the other rats.

The overall relationship between sodium intake and SNGFR distribution is depicted in Figure 1. All rats are shown individually and grouped according to sodium intake. Special symbols identify rats from the same litter. The S/JM SNGFR ratio was remarkably independent of the level of sodium intake.

Figure 2 depicts the correlation between the summated SNGFR in each study and the corresponding inulin clearance for all the rats included.
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FIGURE 2

Relationship between the stimulated single nephron glomerular filtration rate (S SNGFR) and the inulin clearance (CIN) in each rat. The line is the line of identity. Symbols are the same as in Figure 1.

Discussion

Because of the structural diversities among nephrons, the composition of the fluid emerging from them could be different (1). Therefore, redistribution of load among nephrons may modify the final composition of the urine. This mechanism has been thought to participate in the regulation of sodium balance (2, 3). The evidence gathered in the present study, however, does not support this view, at least with respect to the daily regulation of sodium excretion in response to a changing sodium intake. The present results are particularly at variance with the dramatic changes in the intrarenal SNGFR distribution in response to a varying dietary sodium intake observed by Horster and Thurau (3). It is, therefore, of special interest to analyze the possible reasons for the discrepant results. Although every effort was made to duplicate the experimental procedure used by Horster and Thurau, some differences were unavoidable. The SNGFR was determined by micropuncture in that study and by a modification of the Hanssen technique (8) in this study. Two drawbacks of the micropuncture technique that need not be duplicated are that the kidney must remain exposed throughout the study and that, to gain access to JM nephrons, the renal pelvis must be opened. These maneuvers may have introduced stimuli to which the reactivity of the kidney varied depending on the level of dietary sodium intake. In the present study the abdominal cavity was closed after placing the tie around the renal pedicle and remained closed throughout the study until after the pedicle was ligated. However, a drawback of the Hanssen technique as used in this study is the effect of ferrocyanide on arterial blood pressure (8, 11) and renal blood flow (11), which could have introduced an acute redistribution of SNGFR capable of overriding preexisting differences created by the various sodium intakes. In the present study, ferrocyanide induced a significant fall in arterial blood pressure averaging 5.8 ± 1.6 mm Hg (P < 0.005). However, in several rats, some of them littermates on high- and low-sodium intake, the fall was minimal or absent (Tables 1 and 2). There was no correlation between the magnitude of the blood pressure fall and the S/JM ratio (r = 0.34, P > 0.10). Moreover, as previously discussed (8), indirect evidence suggests that the systemic vascular effects of intravenously administered ferrocyanide, when moderate, do not alter the SNGFR distribution. The rationale for this argument is based on the similarity of the S/JM SNGFR ratios obtained with two different modifications of the Hanssen technique, one of which (12) avoids the drop in arterial blood pressure during the SNGFR measurement.

The mean SNGFR for S, intermediate (1), and JM nephrons was similar in kidneys frozen in situ immediately after tying the pedicle and in those frozen after some delay (Table 3). This observation suggests that there was no detectable formation of filtrate after the pedicle was tied, presumably because the filtration pressure gradient was quickly dissipated. Moreover, the data also suggest that there was no reverse filtration or bulk passage of ferrocyanide molecules with water from Bowman’s capsule back into the glomerular capillaries and out of the glomerulus, presumably because little plasma remained in the glomerular capillaries after cessation of arterial input. Diffusion of ferrocyanide molecules was not expected, because there is concentration equilibrium across the filtering membrane. Also in support of these conclusions is the
TABLE 3

Effect of Time Elapsed between Tying and Freezing on SNGFR (uliters/min 100 g⁻¹ body weight)

<table>
<thead>
<tr>
<th>Time</th>
<th>Superficial nephron</th>
<th>Intermediate nephron</th>
<th>Juxtamedullary nephron</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–30 seconds (16)</td>
<td>14.0 ± 0.6</td>
<td>14.0 ± 0.7</td>
<td>17.5 ± 0.7</td>
</tr>
<tr>
<td>1–3 seconds (8)</td>
<td>13.3 ± 0.7</td>
<td>14.3 ± 0.9</td>
<td>18.9 ± 1.6</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent the number of rats in each group. NS = not significant.

agreement between Σ SNGFR and inulin clearance regardless of the delay in freezing (Fig. 1).

Another potential source of difference with the work of Horster and Thurau (3) concerns the nephron sampling. The nephrons punctured at the tip of the papilla undoubtedly were JM nephrons, but it is possible that they belonged to a special subpopulation not representative of the true JM population. In support of this possibility is the fact that the mean SNGFR in punctured JM nephrons of rats kept on a low-sodium (3) or a standard-sodium (13) intake was higher than that measured by the quantitative modifications of the Hanssen technique (8, 12). However, if these nephrons had a higher SNGFR because they were larger and reacted to a high-sodium intake with a 72% drop in SNGFR, then nephrons from this special subpopulation could be found among all the microdissected JM nephrons. In other words, there should be a tendency in rats on a high-sodium intake for the larger JM nephrons to have a lower SNGFR. In the present study, a total of 118 JM nephrons was microdissected from the ten rats on a high-sodium intake. To equalize the data, the individual proximal tubular lengths and the values of SNGFR were factored by the mean S proximal length and SNGFR, respectively, in each rat. The plot of the equalized lengths and the values of SNGFR (Fig. 3) shows no tendency for the larger nephrons to have a lower SNGFR. The least-squares regression line has a positive slope significantly different from zero (P < 0.001). It is unlikely, therefore, that the JM nephrons accessible to micropuncture represent a subpopulation of JM nephrons exhibiting a different physiological response to sodium intake. At any rate, such a subpopulation would be too small to have physiological significance.

Since the data in Figure 3 show that there is a significant, although weak, correlation between the length of the JM proximal tubule and its SNGFR (r = 0.40, P < 0.001), one must guard against the possibility of variations in the S/JM SNGFR ratio for a given rat introduced by differences in the mean length of the nephrons sampled. It is particularly important that no distortion be introduced by this factor in the comparison between groups with different sodium intakes. This possibility was examined by plotting the S/JM ratio of proximal tubular length against that of SNGFR for each rat. Figure 4 shows that there is a significant, positive correlation between these two parameters (r = 0.70, P < 0.001), i.e., as the two nephron populations approach each other in mean length, so do the corresponding mean values of SNGFR, and vice versa. However, there is no difference between rats on low- and high-sodium intake. This figure suggests that the two high S/JM SNGFR ratios in the group with a sodium intake of 3 mEq/day (Fig. 2) are attributable to the fact that JM nephrons in these two rats were closer in size to the corresponding S nephrons.

The present results are also at variance with those of de Rouffignac and Bonvalet (6), who found a
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moderate redistribution of SNGFR toward S nephrons during high dietary sodium intake as measured by the Hanssen technique. However, the physiological state of their animals on high-sodium intake differed significantly from that of rats studied in this paper, as evinced by the markedly increased urine flow and inulin clearance. This difference can be attributed to a rapid hypertonic saline infusion, which undoubtedly caused an expansion of extracellular fluid volume and some degree of hemodilution. Redistribution of SNGFR toward S nephrons following acute saline loading in the rat has been reported (4, 7, 14). In the study of de Rouffignac and Bonvalet (6) there was no difference in SNGFR distribution between animals on low- and standard-sodium diets, presumably involving a severalfold change in sodium intake, but without an acute saline load. Similarly, there was no difference in SNGFR distribution between two micropuncture studies (3, 13) in spite of a fourfold difference in sodium intake, judging from the reported composition of the respective low-salt (3) and standard (13) diets. Finally, in one study there was no difference in SNGFR between two groups on high-sodium diets, in spite of a twofold difference in sodium intake (3).

Recently, Baines (7) suggested that redistribution of SNGFR might only occur in young rats and proposed a structural rather than a functional basis for redistribution. In the present study, most rats had body weights above 250 g, a range where a lack of redistribution would be predicted. Thus, our results do not contradict this interpretation. However, the few rats with lower body weights also failed to undergo redistribution (Tables 1 and 2), and there was no relationship between body weight and the S/JM SNGFR ratio in rats on high- or low-sodium intake (Fig. 5) over a range extending from 157 to 395 g. If redistribution does take place in younger rats, the data from Baines (7) indicate that it is of small magnitude and not related to the natriuresis.

It may be concluded from the present data that the wide variations in dietary sodium intake tested did not give rise to changes in the intrarenal SNGFR distribution or did so to an undetectable extent. The absence of SNGFR redistribution does not preclude the existence of a renal blood flow redistribution, detected in man in association with changes in dietary sodium intake (15, 16), which could participate in the regulation of sodium excretion even without SNGFR redistribution through the corresponding changes in filtration fraction (17). Recent studies in the rat, however, suggest that variations in dietary sodium intake do not affect the intrarenal blood flow distribution (18). Therefore, the theoretical advantages of nephron heterogeneity, in terms of additional functional flexibility, do not materialize or are not called on to participate in the chronic adjustments...

FIGURE 4
Effect of the distribution of nephron lengths on the single nephron glomerular filtration rate (SNGFR) distribution. Symbols are the same as in Figure 1. Regression line calculated by least squares: $y = 0.094x - 0.06$, $r = 0.070$, $P < 0.001$. S = superficial nephrons, and JM = juxtamedullary nephrons.
of sodium excretion required to maintain sodium balance in the rat.

Acknowledgment
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References
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