Original Contributions

Evidence for Increased Renal Norepinephrine Overflow During Sodium Restriction in Humans

Peter Friberg, Ian Meredith, Garry Jennings, Gavin Lambert, Virginia Fazio, and Murray Esler

To investigate the differentiated pattern of efferent sympathetic nerve activity by means of analyzing norepinephrine kinetics in response to sodium restriction, cardio-renal sympathetic activity during rest and mental stress was studied in 12 subjects (33.3±2.6 years old, SEM) exposed to a low and a normal sodium diet; 5-40 mmol and 160-200 mmol/24 hours, respectively (crossover design). Organ norepinephrine release was calculated from organ plasma flow, arteriovenous plasma concentration gradient across the organ and the organ's fractional extraction of radiolabeled norepinephrine. Body weight and urinary sodium/24 hr fell significantly and urinary potassium/24 hr and both supine and standing blood pressure remained unchanged. Total norepinephrine release to plasma and norepinephrine plasma clearance were similar in both phases (≈460 ng/min and 1.90 l/min, respectively). A 138% increase in renal norepinephrine overflow was observed during sodium restriction (from 112 to 267 ng/min, p<0.025), which was due to elevated renal vein norepinephrine (434 versus 290 pg/ml, p<0.01) because renal plasma flow and renal norepinephrine extraction were unaltered. Similarly, sodium restriction caused a 168% elevation of renal renin secretion (p<0.05). Resting cardiac norepinephrine spillover and cardiac norepinephrine reuptake were unchanged between the two salt phases. Total and cardiac norepinephrine release, supine blood pressure, and heart rate increased to about the same extent in response to mental testing regardless of salt phase. In conclusion, sodium restriction induced a differential and physiological increase in resting renal sympathetic nervous activity, leaving cardiac norepinephrine overflow unchanged. Cardiac norepinephrine uptake was normal, which further supports the concept of a true increase of efferent nerve activity. (Hypertension 1990;16:121-130)

The various control mechanisms securing the sodium content of the organism usually adjust intake, absorption, and excretion so efficiently that the internal sodium equilibrium is maintained largely intact even during severe deprivation or excess. Most experimental and clinical work has been focused on the relations between blood pressure and sodium intake. However, knowledge of underlying regulatory mechanisms is still insufficient.

During dietary sodium restriction, several neurohormonal and hemodynamic mechanisms are activated to reduce daily urinary sodium excretion. Several studies have shown elevations of plasma norepinephrine (p-NE) concentration, suggestive of sympathetic nervous system activation, in response to short periods of sodium restriction, whereas others have demonstrated no difference. Various levels of 24-hour sodium excretion, age differences, and different venous or arterial sampling sites for NE are some major factors underlying the contradictory results.

p-NE concentration is influenced not only by spillover of NE from the organs into the blood but also by its clearance. Using NE kinetics in sodium-restricted healthy subjects, by means of infusing [H]NE, Watson et al demonstrated an elevation of total p-NE spillover rate and an unaltered p-NE clearance. As pointed out by Folkow and coworkers, sympathetic nerve-fiber activation often occurs in a differentiated way as, for example, with the defense reaction.
Consequently, it is of great relevance to make detailed regional analyses of NE overflow into different organ vascular circuits.14

Whether the increase in total sympathetic nerve fiber discharge in response to sodium restriction as observed by Watson et al12 was a generalized phenomenon or if it represents a regional response is not known. The sympathetic nervous system influences many aspects of kidney function, particularly during states of sodium deprivation, where renal vascular tone, intrarenal renin release, and proximal and distal tubular reabsorption of electrolytes and fluid are all likely to be augmented.15 Renal nerve function is essential for an organism exposed to sodium depletion to maintain homeostatic sodium balance by means of directly increasing sodium reabsorption, in part, through sympathetically mediated intrarenal release (β2-adrenergic and α1-adrenergic receptors).16 DiBona and Sawin17 showed the importance of the renal sympathetic nerves for maintenance of stable plasma sodium concentrations by demonstrating a progressive negative sodium balance after bilateral renal denervation despite the fact that the rats were exposed to a low sodium diet. Moreover, in sodium-restricted rats and dogs, an increase was found in renal nerve activity and renal vein NE, respectively, compared with animals fed a normal sodium diet,7 indicating an augmentation of renal sympathetic activity. On the basis of this, one of the aims of the present study was to explore whether a selective sympathetic activation of the kidneys occurs in healthy subjects exposed to a short-term sodium restriction. This was examined by determining renal and cardiac NE spillover rate by means of selective venous and arterial sampling from the kidney and the heart, respectively. Furthermore, it has been suggested that pressor responses to mental arithmetic are reduced in hypertensive patients as a consequence of a low sodium diet.19 Blood pressure and heart rate responses as well as cardiac NE spillover measurements were therefore analyzed during rest and arithmetic tests to validate whether normotensive healthy subjects fed a low sodium diet were less responsive with regard to sympathetic activation.

Neuronal uptake (uptake-1) has been shown to be the greatest contributor to removal of NE from the synaptic cleft, at least in the forearm20 and heart.21 To date no data exist in humans demonstrating neuronal uptake changes during sodium restriction. To address this question, we measured cardiac NE spillover and clearance after an infusion of the uptake-1 inhibitor desipramine.

Methods

Experimental Subjects

Fourteen healthy male volunteers (mean age 33.3±2.6 years old [SEM], range 25–50) participated in the study, which was approved by the Alfred Hospital Medical Research Ethics Committee. All participants were fully informed and gave their written consent. Subjects were recruited by advertisement from the general community and were studied as outpatients. No subject showed evidence of cardiovascular or other disease based on medical history, physical examination, and routine laboratory tests. None of the volunteers took any medication either at the time of the study or for at least 1 month before entering the study.

Experimental Design

The investigation was a longitudinal one with a crossover design, consisting of one low salt phase (12.9±0.6 days long) and one normal salt phase (13.2±1.4 days long). Sodium balance was then well established. There was an initial period during which screening and education were performed. A low sodium intake was achieved by continuous instruction from a dietitian at the Alfred Hospital. The sodium intake was limited to <40 mmol/24 hr during both phases, and to normalize sodium intake (during normal salt phase), sodium chloride gelatin capsules (1.5 g) were administered five times daily (134 mmol/24 hr). Some subjects reported nausea after capsule intake, and they were advised to open the capsules and spread the sodium chloride over food. Compliance was assessed by measuring 24-hour urinary electrolyte excretion (U-Na/24 hr) at least three times during each phase. To be included in the study, sodium excretion was not allowed to exceed 40 mmol/24 hr on the low salt catheter day. Other nutrients and calories were kept constant throughout the study. Carbohydrates constituted approximately 45–55% of dietary intake, 30–35% was fat, and 12–16% was protein. The low salt diet halved the amounts of calcium, iron chloride, and zinc, whereas potassium intake remained unchanged. Most of the subjects went through both phases, and the order of the initial phase was randomized. In some subjects supraventricular arrhythmia occurred; hence there were some subjects who did not follow the study to the end (see Table 1 for details).

At each visit, urinary electrolytes, supine and standing blood pressure, heart rate, and body weight were measured. Supine blood pressure and heart rate recordings were performed after 20 minutes of quiet rest and standing values after 5 minutes. Two nurses were assigned to perform the blood pressure measurements, which were obtained using a random zero sphygmomanometer.

Experimental Procedures

In the morning of the final day of each diet period, subjects were given a standardized low salt breakfast with or without sodium chloride capsules, depending on prevailing phase. Coffee, tea, alcohol, and tobacco were prohibited for 12 hours before each study day. On each study day, insertion of catheters was done under strict aseptic conditions using local anesthesia. A 21-gauge cannula was introduced percutaneously into either the left or right brachial artery for blood pressure monitoring and blood sampling, and in the
same arm, an 8.5F percutaneous introducing sheath was inserted into the median antecubital vein. Selective coronary sinus and right renal vein catheterization were performed via the forearm venous sheath using a 7F coronary sinus thermodilution catheter (Webster lab, USA type CCS-70) and 7F 130 cm Cournand catheter, respectively. Catheterization was carried out under direct fluoroscopic control and correct positioning of catheters verified with 2 ml radiopaque contrast medium (Omnipaque, Winthrop Pharmaceuticals, New York). In the other arm, para-aminohippurate (PAH) and l-[^3H]NE (New England Nuclear, Boston, Mass.) were infused into a peripheral vein via a 23-gauge peripheral intravenous line. PAH was infused, after a priming bolus of 120 mg, at a rate of 5 mg/min/m². Coronary sinus blood flow was measured as the mean of several determinations by means of thermodilution, with rapid infusions of dextrose at room temperature.²² For the determination of renal plasma flow, standard curves relating optical density to the concentration of PAH in duplicate experimental samples were determined from the curves and used to calculate infusion clearance, which was corrected for fractional extraction to derive plasma flow. Blood and plasma flows were interconverted using the subject's hematocrit level at the time.

**Norepinephrine Kinetics**

A tracer dose of [7-[^3H]NE (specific activity 14–22 Ci/mmol) was infused at constant rate (0.70 μCi/min) for approximately 80 minutes for measurement of the apparent release rate of NE into plasma and clearance of NE from plasma.²³,²⁴ After reaching steady-state conditions, the rate of release (spillover rate) of endogenous NE into plasma and NE clearance from plasma was determined²³,²⁴ using the following equations:

\[
\text{Norepinephrine spillover rate} = \frac{\text{norepinephrine infusion rate}}{\text{specific radioactivity of plasma norepinephrine}}
\]

(1)

\[
\text{[^3H]Norepinephrine plasma clearance} = \frac{\text{norepinephrine infusion rate/plasma [^3H]norepinephrine concentration}}{}
\]

(2)

The assessment of sympathetic nervous activity in individual organs from measurements of the rate of spillover of released NE into the venous drainage is
were obtained, rapidly followed by simultaneous coronary sinus flows of 10 minutes of mental arithmetic, coronary sinus and blood sampling was repeated. The subject was then exposed to a 10-minute test of mental arithmetic, subtracting either 13 or 17 from a three-digit number. The test was performed once before the first catheter day to habituate the subject. The venous catheter preceded blood sampling. Before the second repositioning of the catheter, arterial epinephrine level was 57.3 ±11.4 pg/ml, indicating a non-stressed experimental situation. The venous catheter was then repositioned from the right renal vein to the coronary sinus and blood sampling was repeated. Arterial pressure and heart rate values had returned to resting levels. The uptake-1 inhibitor desipramine was then infused over a period of 30 minutes (0.5 mg/kg) followed by coronary sinus and arterial blood sampling.

Biochemical Measurements

All blood samples for catecholamine measurements were collected on ice into test tubes containing reduced glutathione, centrifuged at 4° C for 20 minutes, and plasma was stored at −30° C for subsequent assay within 1 month. The endogenous p-NE concentration was measured in duplicate by the method of Peuler and Johnson,27 and plasma tritiated NE was calculated by liquid scintillation counting after alumina extraction. Sensitivity of the radioenzymatic assay for NE was 10–15 pg/ml, and the coefficient of variation between assays was 10%. Plasma renin activity (renal vein and arterial) was measured by enzymatic generation of angiotensin I, followed by radioimmunoassay of the generated angiotensin I.28

Statistics

Results are expressed as mean±SED for intergroup comparisons. Statistical significance was assessed by the Student’s t test for paired observations. Additionally, analysis of variance and partitioning were performed.29 A p value of less than 0.05 was considered statistically significant.

Results

As shown in Table 2, there was a small but significant decrease in body weight with salt restrictions. A substantial fall in U-Na/24 hr level was evident during the low salt phase, whereas U-K/24 hr level did not change. U-Creat/24 hr, urinary sodium, potassium, and creatinine excretion during 24 hours, respectively; SED, standard error of the difference; p<, indicates probability level; and NS, indicates no significant difference.

<table>
<thead>
<tr>
<th>Salt phase</th>
<th>Body weight (kg)</th>
<th>U-Na/24 hr (mmol)</th>
<th>U-K/24 hr (mmol)</th>
<th>U-creat/24 hr (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt phase (n=10)</td>
<td>76.3 (65.4–86.8)</td>
<td>152 (119–261)</td>
<td>84</td>
<td>15.1</td>
</tr>
<tr>
<td>Low salt phase (n=10)</td>
<td>75.5 (63.9–86.8)</td>
<td>35 (5–39)</td>
<td>76</td>
<td>13.8</td>
</tr>
<tr>
<td>SED</td>
<td>0.2</td>
<td>9</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>p&lt;</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mean values are obtained from the three clinical visits during either normal salt or low salt phase. Statistical analysis was performed by means of two-way analysis of variance and partitioning. In separate analyses it was shown that the order of visits to the clinic did not influence the results. Measured differences were only due to change of salt phase. U-Na/24 hr, U-K/24 hr, U-creat/24 hr, urinary sodium, potassium, and creatinine excretion during 24 hours, respectively; SED, standard error of the difference; p<, indicates probability level; and NS, indicates no significant difference.

Complicated by the fact that all organs release NE into and remove NE from the circulation simultaneously.25-26 Accordingly, NE spillover cannot be estimated from measurements of venoarterial plasma NE concentration differences and organ blood flow alone. Because NE flux is bidirectional, determination of NE spillover rates by the Fick principle requires, in addition, the simultaneous measurement of NE extraction. NE extraction is probably best determined by using a constant rate infusion of isotope-labeled NE25:

\[ \text{Organ norepinephrine spillover} = \left( C_v - C_a \right) \times \frac{C_e \times \text{NE}_e}{\text{PF}} \]  

where \( C_v \) is plasma NE concentration in draining vein, \( C_a \) is arterial plasma NE concentration, \( \text{NE}_e \) is fractional extraction of tritiated NE, and PF is organ plasma flow. Renal and cardiac NE spillover were calculated from the arteriovenous plasma NE concentration difference (with renal vein and coronary sinus sampling), the arteriovenous difference in NE by the kidney and heart could be calculated, and the respective organ flows as per equation.3

**Study Protocol**

During rest one paired arterial and right renal vein blood sample was obtained approximately 80 minutes after starting the tritiated NE infusion. A minimum of 10 minutes of quiet rest after placement of the catheters preceded blood sampling. Before the second repositioning of the catheter, arterial epinephrine level was 57.3 ±11.4 pg/ml, indicating a non-stressed experimental situation. The venous catheter was then repositioned from the right renal vein to the coronary sinus and blood sampling was repeated. Arterial pressure and heart rate were also monitored. The subject was then exposed to a 10-minute test of mental arithmetic, subtracting either 13 or 17 from a three-digit number. The test was performed once before the first catheter day to habituate the subject. The person who performed the mental arithmetic did not know the current salt phase. After about 8 minutes of mental arithmetic, coronary sinus flows were obtained, rapidly followed by simultaneous coronary sinus and arterial blood sampling. Ten minutes later, arterial pressure and heart rate values had returned to resting levels. The uptake-1 inhibitor desipramine was then infused over a period of 30 minutes (0.5 mg/kg) followed by coronary sinus and arterial blood sampling.

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All blood samples for catecholamine measurements were collected on ice into test tubes containing reduced glutathione, centrifuged at 4° C for 20 minutes, and plasma was stored at −30° C for subsequent assay within 1 month. The endogenous p-NE concentration was measured in duplicate by the method of Peuler and Johnson,27 and plasma tritiated NE was calculated by liquid scintillation counting after alumina extraction. Sensitivity of the radioenzymatic assay for NE was 10–15 pg/ml, and the coefficient of variation between assays was 10%. Plasma renin activity (renal vein and arterial) was measured by enzymatic generation of angiotensin I, followed by radioimmunoassay of the generated angiotensin I.28

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**Results**

As shown in Table 2, there was a small but significant decrease in body weight with salt restrictions. A substantial fall in U-Na/24 hr level was evident during the low salt phase, whereas U-K/24 hr level did not change. U-Creat/24 hr was slightly reduced. Supine and standing blood pressures remained unchanged with sodium restriction, but both supine and standing heart rates were elevated during the low salt diet (Table 3). When measuring intraarterial mean arterial pressure on the low salt catheter day, however, it was found to be reduced compared with the normal salt catheter day (90 versus 100 mm Hg, SED±3, p<0.005). Heart rate did

**TABLE 2. Body Weight and Urinary Electrolytes in Ten Healthy Subjects Exposed to Normal and Low Sodium Diets in a Random Order (Crossover Design)**

<table>
<thead>
<tr>
<th>Salt phase</th>
<th>Body weight (kg)</th>
<th>U-Na/24 hr (mmol)</th>
<th>U-K/24 hr (mmol)</th>
<th>U-creat/24 hr (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>15.1</td>
</tr>
<tr>
<td>Low salt phase</td>
<td>75.5 (63.9–86.8)</td>
<td>35 (5–39)</td>
<td>76</td>
<td>13.8</td>
</tr>
<tr>
<td>SED</td>
<td>0.2</td>
<td>9</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>p&lt;</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mean values are obtained from the three clinical visits during either normal salt or low salt phase. Statistical analysis was performed by means of two-way analysis of variance and partitioning. In separate analyses it was shown that the order of visits to the clinic did not influence the results. Measured differences were only due to change of salt phase. U-Na/24 hr, U-K/24 hr, U-creat/24 hr, urinary sodium, potassium, and creatinine excretion during 24 hours, respectively; SED, standard error of the difference; p<, indicates probability level; and NS, indicates no significant difference.
Accordingly, that subject did not show an elevation of calcium levels, respectively; SED, standard error of the difference.

**Table 4.** Serum Electrolytes Obtained at End of Each Salt Phase at the Time of Catheterization

<table>
<thead>
<tr>
<th>Salt phase</th>
<th>S-Na (mmol/l)</th>
<th>S-K (mmol/l)</th>
<th>S-Ca (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt phase (n=10)</td>
<td>140</td>
<td>4.0</td>
<td>2.25</td>
</tr>
<tr>
<td>Low salt phase (n=10)</td>
<td>139</td>
<td>4.1</td>
<td>2.28</td>
</tr>
<tr>
<td>SED</td>
<td>1</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>p&lt;</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean values. S-Na, S-K, S-Ca, serum sodium, potassium, and calcium levels, respectively; SED, standard error of the difference; p<, indicates probability level; NS, indicates no significant difference.

not differ between catheter days. Hematocrit did not change with sodium restriction and was 41.8% for the normal salt phase and 41.6% for the low salt phase, respectively (SED±0.84). Furthermore, levels of the three serum electrolytes sodium, potassium, and calcium were unchanged during the low sodium diet (Table 4).

Table 1 presents the individual catheterization protocol and shows the reasons not all of the fourteen subjects initially enrolled in the study continued to the end. For example, two subjects were excluded because atrial fibrillation had occurred on the first catheter day before blood sampling. Neither arterial p-NE concentration nor clearance of p-NE changed significantly with sodium restriction (Table 5). Thus, total NE spillover rate to plasma was unchanged.

After a period of sodium restriction, p-NE concentration in the renal vein increased significantly (Table 6). Because fractional extraction of tritiated NE across the kidney and renal plasma flow were unchanged during sodium restriction, it follows that renal NE spillover rate increased markedly in the low salt state (Table 6 and Figure 1, top panel). It can also be seen in Figure 1 that renal NE spillover rate is rather dependent on the level of 24-hour urinary sodium excretions (numbers in brackets). All subjects except one (dotted line in Figure 1) had a daily urinary sodium excretion of less than 40 mmol. Accordingly, that subject did not show an elevation of renal NE spillover rate but was not included in the subsequent calculations. Figure 2 shows that renal NE spillover rate more than doubled in the low salt phase, constituting about 58% of total spillover rate. In fact, extrarenal NE spillover has decreased in subjects during sodium restriction compared with the normal sodium phase.

Both arterial and renal vein plasma renin activity increased with sodium restriction (Table 7). Thus, renal renin secretion increased significantly.

Calculated renal vascular resistance did not change significantly between the low and the normal salt phase (54 and 62 mm Hg/min·m−1·SED±6, NS).

Coronary sinus plasma flows were approximately 80 ml/min and were not significantly different between the two phases. Likewise, mean fractional extraction of tritiated NE across the heart was approximately 56% in both phases.

Cardiac NE spillover rate did not change with sodium restriction (individual data in Figure 1, bottom panel) and was 14.2 and 13.0 ng/min for low and normal salt, respectively (SED±2.2, NS).

After desipramine infusion, extraction of NE across the heart fell to the same extent (56% to 14%) in both phases (Figure 3). Cardiac NE spillover rate increased from 9.8 to 23.7 ng/min (SED±4.5, p<0.01) during the normal salt phase and from 8.7 to 19.1 ng/min (SED±3.2, p<0.01) during sodium restriction (see Figure 3). Total NE spillover rate fell in response to desipramine both during the normal salt phase (from 461.4 to 263.8 ng/min, SED±79.0, p<0.05), and during the low salt phase (from 426.9 to 300.9 ng/min, SED±82.7), although not statistically significant in the latter. Additionally, total NE clearance fell significantly during the sodium supplemen-

**Table 5.** Total Norepinephrine Kinetics Measured in Arterial Blood From Subjects Fed Normal and Low Sodium Diets

<table>
<thead>
<tr>
<th>Salt phase</th>
<th>Plasma NE (pg/ml)</th>
<th>Plasma NE clearance (l/min)</th>
<th>Total NE spillover rate (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt phase</td>
<td>233</td>
<td>2.02</td>
<td>463</td>
</tr>
<tr>
<td>Low salt phase</td>
<td>262</td>
<td>1.83</td>
<td>462</td>
</tr>
<tr>
<td>SED</td>
<td>18</td>
<td>0.11</td>
<td>56</td>
</tr>
<tr>
<td>p&lt;</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean values. NE, norepinephrine; SED, standard error of the difference; p<, indicates probability level; NS, no significant difference.
TABLE 6. Renal Norepinephrine Kinetics Measured in Paired Blood Samples From the Brachial Artery and Right Renal Vein in Subjects Fed Normal and Low Sodium Diets

<table>
<thead>
<tr>
<th>Salt phase</th>
<th>A-NE (pg/ml)</th>
<th>RV-NE (pg/ml)</th>
<th>[3H]NE extraction (%</th>
<th>RPF (ml/min)</th>
<th>Renal NE spillover rate (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt phase</td>
<td>233</td>
<td>290</td>
<td>38</td>
<td>994</td>
<td>112</td>
</tr>
<tr>
<td>Low salt phase</td>
<td>262</td>
<td>434</td>
<td>41</td>
<td>954</td>
<td>267</td>
</tr>
<tr>
<td>SED</td>
<td>18</td>
<td>38</td>
<td>3</td>
<td>83</td>
<td>47</td>
</tr>
<tr>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Mean values. A, brachial artery; NE, norepinephrine; RV, right renal vein; RPF, renal plasma flow; SED, standard error of the difference; p<, indicates probability level; NS, no significant difference.

Discussion

The present study demonstrates a clear differentiation in efferent sympathetic nervous system activity as reflected by the spillover rate of NE to plasma after sodium restriction in normotensive subjects. During a controlled period of sodium deprivation (<40 mmol/24 hr of U-NA), renal spillover rate of NE increased by some 140%, whereas cardiac spillover rate of NE remained unchanged. The results therefore stress the importance of NE spillover rate measurements in individual organs, in this case with emphasis on the kidneys because of their involvement in salt and water regulation. Even at low sodium intake, salt and water regulation showed a clear increase of NE spillover, even though total spillover rate of NE was unchanged. The rate of NE escape into the renal venous drainage is largely proportional to the rate of regional sympathetic discharge, as indicated in the dog.30,31 One should bear in mind, however, that a variety of other factors

![Figure 1](image1.png)

![Figure 2](image2.png)
TABLE 7. Plasma Renin Activities Measured in Paired Blood Samples From the Brachial Artery and Right Renal Vein in Subjects Fed Normal and Low Sodium Diets

<table>
<thead>
<tr>
<th>Salt phase</th>
<th>PRA-arterial (ng/ml/hr)</th>
<th>PRA-renal vein (ng/ml/hr)</th>
<th>Renal renin secretion (ng/ml/hr/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt phase</td>
<td>0.46</td>
<td>0.56</td>
<td>81</td>
</tr>
<tr>
<td>Low salt phase</td>
<td>0.74</td>
<td>0.97</td>
<td>217</td>
</tr>
<tr>
<td>SED</td>
<td>0.08</td>
<td>0.08</td>
<td>55</td>
</tr>
<tr>
<td>p&lt;</td>
<td>0.01</td>
<td>0.005</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mean values. PRA, plasma renin activity; SED, standard error of the difference; p<; indicates probability level.

FIGURE 3. Plots showing effects of neuronal uptake blockade of norepinephrine (noradrenaline), determined by a 25–30-minute infusion of antidepressant tricyclic desipramine (DMI) (0.5 mg/kg) on total norepinephrine clearance, cardiac extraction of tritiated norepinephrine, norepinephrine spillover, mean arterial pressure, and heart rate in individual subjects exposed to normal and low sodium diets. Note increases in heart rate and mean arterial pressure after infusion. Statistical evaluation was based on those subjects that went through the whole procedure with DMI infusion at two occasions (six for norepinephrine spillover measurements and seven for the other parameters). n.s., not significant.

FIGURE 4. Plots showing total and cardiac norepinephrine (noradrenaline) spillover rates and mean arterial pressure and heart rate during 10 minutes of mental arithmetic in subjects receiving normal and low sodium diets. Values are mean±SEM. Solid lines represent normal sodium phase and dotted lines low sodium phase. n.s., not significant.

(e.g., the neurogenic reduction of renal plasma flow) may influence renal NE spillover (and that of other organs) into plasma. However, it has been shown in the dog that only pronounced renal blood flow reductions affect NE spillover to plasma, and in the present study, renal plasma flow was the same at low and normal salt intakes.

A number of studies have tried to evaluate "sympathetic activity" by merely analyzing changes in plasma NE in response to sodium restriction (see above). For different reasons, more or less contradictory results have been obtained. The usefulness of p-NE concentrations alone as an index of sympathetic activity is, however, limited because plasma NE concentrations are determined by the rates of both entry into plasma and its removal. In accordance with a previous study, no change in NE clearance was observed in the present study. However, previous work noted an increased total NE spillover rate, but this parameter was unchanged in the present experiments. The difference might be due to more complicated catheterization procedures in our study or the sampling site; Watson et al took antecubital venous blood (draining mostly muscle and skin), whereas in the present study arterial sampling was performed. Further, the subjects herein had a slightly higher daily U-Na excretion than the subjects presented by Watson and coworkers.
would expect these receptors to reset after obtaining partially secondary to the increased sympathetic mechanisms may come into play later in the sodium-restricted phase. This implies some reduction of NE release from other organs, perhaps the skeletal muscles. Among the multiplicity of adaptations to sodium deprivation, it is difficult to know what could be the immediate trigger for the elevated renal NE release. Several mechanisms for an increased renal NE spillover rate could be considered.

First, a reduced extracellular fluid volume, reflected as a decreased right atrial pressure, might, through central cardiopulmonary receptors, more or less selectively activate splanchnic (renal) sympathetic activity. However, in the present sodium-restriction phase, the renal NE spillover contribution to total NE spillover was 58%, which corresponds to a doubling compared with the normal salt phase. Because the total NE spillover rate was the same in both phases, this implies some reduction of NE release from other organs, perhaps the skeletal muscles. Among the multiplicity of adaptations to sodium deprivation, it is difficult to know what could be the immediate trigger for the elevated renal NE release. Several mechanisms for an increased renal NE spillover rate could be considered.

Second, sodium restriction might cause changes in activity in central nervous system neurons, which in turn selectively may increase renal sympathetic activity, as cardiac NE spillover rate was unaffected by sodium restriction.

Third, it is common knowledge that plasma renin activity increases with sodium restriction. This is partially secondary to the increased sympathetic activity. Further, released angiotensin II could, in turn, facilitate NE release in the kidney and potentially the effects of NE postsynaptically, although in the present study, regardless of salt phase, there was no difference in angiotensin II concentrations (about 10–15 pg/ml) in the renal vein or arterial blood. These values have to be taken with great caution, because the sensitivity of the assay was approximately 10 pg/ml. In any case, there was no increase in angiotensin II production from the kidney during sodium restriction.

The level of sodium intake (and excretion) seems to be of great relevance for renal NE spillover rate (i.e., for the level of renal sympathetic activity). Figure 1 shows individual data of renal NE spillover and sodium excretion levels in brackets. All subjects below the excretion level of 40 mmol/24 hr (inclusion criterion) demonstrated an increased NE spillover rate during sodium restriction. However, one subject failed to reduce sodium intake during the 24 hours preceding the examination, and in this subject, the renal NE spillover rate remained unaltered compared with the rate during normal sodium intake.

Sodium restriction in our study, which increased renal NE spillover rate, did not reduce renal vascular resistance. This is somewhat puzzling, because the prevailing high levels of NE and plasma renin activity did not at all influence vascular resistance. However, previous data have shown that sodium restriction also increased the levels of urinary kallikrein and prostaglandin E2, which are both potent vasodilators. Neither systolic nor diastolic blood pressures (supine and standing) changed in response to sodium restriction, whereas both supine and standing heart rates increased significantly. Because cardiac NE spillover rate remained unchanged after sodium restriction, one cannot exclude reduced vagal tone as a possibility for the elevated heart rate.

The present study further demonstrates that sympathetic responsiveness to mental stimuli in healthy normotensive subjects, as reflected by mean arterial pressure, heart rate, and cardiac NE spillover rate, was similar during both normal and low sodium intakes (see Figure 4), indicating no negative impact on adrenergic responsiveness by sodium restriction as was noticed previously in hypertensive patients.

Esler et al showed recently that untreated hypertensive patients on a free diet responded to mental arithmetic testing with increases of both total (70%) and cardiac (200%) NE spillover rates, whereas mean arterial pressure and heart rate elevations corresponded closely to the present ones. The explanation for the difference in NE spillover rates in the two studies from the same laboratory is unclear. However, the different diets may be a reason. In the present study, subjects received a special sodium-restricted diet, which was supplemented with sodium capsules during the normal salt phase. As indicated above, several other ions were likely to be reduced, regardless of salt phase.
When specific radioactivity is measured in a blood sample from the coronary sinus, it is now believed that roughly 15% of the activity emanates from the tritiated metabolite dihydroxyphenylglycol (DHPG).\textsuperscript{21,39} Thus, with the presently used technique for determining radioactivity where the radioactivity of [\textsuperscript{3}H]DHPG was not accounted for, the fractional extraction of cardiac NE and thus NE spillover were somewhat underestimated. However, all cardiac samples in the present study have this inherent error.

The relatively high incidence of transient atrial fibrillation with no evident link to salt phase that occurred during catheter placement in this study warrants special comment. Atrial fibrillation of short duration occurred on five occasions. In two subjects this preceded sampling for catecholamine measurements (vide supra); results from three subjects in whom atrial fibrillation occurred after sampling were included (Table 1). Several hundred such catheterizations have been performed in our laboratory, and none have resulted in atrial fibrillation. In this study five of 26 of the catheterizations led to atrial fibrillation. It should also be stressed that, simultaneously with the present sodium-restriction study, several other studies were performed involving coronary sinus blood sampling, and none of these subjects went into atrial fibrillation. The mechanism behind this high incidence of arrhythmia in the present study is difficult to explain, but sodium as such does not seem to play a role. Another unplanned aspect of the dietary manipulation may perhaps be involved (dietary calcium and magnesium, for example, were reduced).

To test that there was no change in NE reuptake with sodium restriction, we measured NE spillover rate in the heart before and after desipramine because desipramine affects cardiac NE extraction from about 55% to 20%.\textsuperscript{14-39} Therefore, if any change of NE reuptake occurred on sodium restriction, the heart would be a sensitive organ to detect it (see Reference 39). As shown in Figure 3, cardiac NE extraction fell considerably after desipramine in both salt phases but with no differences between the phases.

Interestingly, in spite of a fall in total NE clearance, the NE spillover rate fell in all organs except the heart, which does not account for a substantial part of total NE spillover rate. Perhaps the low dose desipramine infusion has central effects (\(\alpha\)-blocking effects have been reported in higher doses), thereby reducing sympathetic nerve firing.\textsuperscript{39} It also remains a possibility that the increased mean arterial pressure noted in response to desipramine reflexly lowers sympathetic output. The augmented cardiac NE spillover rate might be explained by the fact that the heart contains an abundance of narrow sympathetic synaptic junctions where a blocked reuptake may have the greatest effects. After desipramine, in line with the increased cardiac NE spillover, mean arterial pressure and heart rate increased in all subjects regardless of salt phase (Figure 3). This suggests that, despite the overall fall in NE spillover, elevated cardiac NE spillover may cause these hemodynamic effects.

In conclusion, the present study has amply illustrated the importance of measuring regional sympathetic activity (with NE-kinetics) instead of only measuring peripheral p-NE, because both p-NE and NE clearance were unchanged during sodium restriction, while renal sympathetic activity increased dramatically. It also stresses the physiological importance of the renal nerves in conserving plasma sodium by minimizing urine sodium losses, possibly by direct renal tubular effects on sodium reabsorption and by stimulating the renin release. The NE reuptake mechanism seems to remain the same during sodium restriction, as tested with the NE uptake-1 blocker desipramine, which further supports the theory of a true increase in renal sympathetic nerve activity.

Moreover, mean arterial pressure, heart rate, and cardiac NE spillover rate increased to about the same degree in response to forced mental arithmetic testing in healthy subjects, thereby excluding any reduced adrenergic responsiveness, at least at the present level of sodium restriction.

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