Effect of acute unilateral renal denervation on tubular sodium reabsorption in the dog

GAKUJI NOMURA, TOSHIKAZU TAKABATAKE, SHIRO ARAI, DENJI UNO, MASATO SHIMAO, AND NOBU HATTORI
First Department of Internal Medicine, School of Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan

The mechanism of "denervation natriuresis and diuresis" is controversial. The bulk of evidence suggests that the natriuresis and diuresis following acute or chronic renal denervation are not related to a change in the glomerular filtration rate (GFR) and/or the renal blood flow (RBF) (2, 4, 5, 7, 16, 17).

Although there are conflicting reports concerning changes in the distribution of intrarenal blood flow as a cause of denervation natriuresis and diuresis, Bencsath et al. (3) and Bellow-Reuss et al. (2) recently showed that there was no redistribution of GFR after denervation in the rat. We have also reported that denervation induced no redistribution of intrarenal blood flow in the dog as judged by the microsphere method. We have suggested that denervation natriuresis and diuresis might be caused primarily by elimination of the nervous control of tubular function (16). In addition, several investigators have shown a direct effect of the renal nerves in the direction of an increase in fractional sodium and water reabsorption (2, 3, 11, 18).

The purpose of this study was to further clarify the site or sites of the nephron involved in denervation natriuresis and diuresis in the dog.

METHODS

Mongrel dogs weighing 7-16 kg were used. Animals were deprived of food but not water for 24 h prior to the study. They were anesthetized with pentobarbital, 30 mg/kg, iv. Small supplementary doses were given thereafter. They were ventilated with a respirator (Harvard Apparatus Co., Millis, Mass.) via an endotracheal tube. The animals were hypophysectomized through a buccal approach with a dental drill and given cortisone hemisuccinate, 1 mg/h, iv.

A cannula was inserted in the femoral artery for blood pressure measurement and blood collection. Each ureter was catheterized with a thin polyethylene tube through a left-flank incision. The left kidney was denervated by sectioning all visible nerves leading to the kidney including those on the renal artery. After all the nerves were cut, the pedicle was painted with 2% Xylocaine.

Renal excretory and clearance data were obtained by means of standard techniques. Priming doses of creatinine (30 mg/kg) and p-aminohippurate (PAH, 8 mg/kg) were given intravenously, after which creatinine (0.57 mg/kg per min) and PAH (0.25 mg/kg per min) dissolved in 0.9% saline were infused intravenously at a constant rate of 1.5 ml/min.

Water diuresis was produced by intravenous infusion of 2.5% dextrose solution, at first 35 ml/kg rapidly, then at 0.4 ml/kg per min. A stable urine flow and a urine osmolality from either kidney below 70 mosmol/kg H2O was the criterion of adequate inhibition of antidiuretic hormone. Three consecutive clearance measurements were then begun with each clearance period 10 min in duration and with blood samples taken in the middle of the period. These clearance measurements usually took place 3-4 h after hypophysectomy and 2-3 h after renal denervation.

Serum and urinary sodium and potassium concentrations were determined with the AutoAnalyzer, creatinine by the method of Bonsnes and Taussky (8), PAH by the method of Brun (9), and osmolality by the osmometer (Fiske Association Inc., Uxbridge, Mass.). Creatinine clearance (Ccr) was used as a measure of glomerular filtration rate and PAH clearance as a measure of effective renal plasma flow (ERPF).
Solute clearance \( (C_{\text{solute}}) \) was calculated as \( U_{\text{solute}} \cdot V / P_{\text{solute}} \), where \( U_{\text{solute}} \) and \( P_{\text{solute}} \) represented urinary and plasma osmolalities respectively, and \( V \) represented urine flow in milliliters per minute. Solute-free water clearance \( (C_{\text{H}_{2}O}) \) was calculated as \( V - C_{\text{solute}} \). Sodium the innervated kidney of all but one dog (Table 1), but the innervated kidney as \( UNa / \left( C_{Na} + C_{H_{2}O} \right) \). The data on urine flow, \( C_{H_{2}O} \), and distal sodium load were expressed per 100 ml GFR in order to obviate differences in GFR from animal to animal.

Results were recorded as means ± standard errors of the means \((\text{means} \pm \text{SE})\). The differences in all parameters between the denervated and the innervated kidney were statistically analyzed by paired-observation t test (14). Results were referred to as significant when their \( \text{P} \) values were \(< 0.01\).

### RESULTS

At the beginning of the clearance measurements, the mean plasma osmolality was 276.7 ± 6.8 mosmol/kg H2O. The mean plasma sodium concentration was 138.3 ± 3.4 meq/liter, and the mean arterial blood pressure was 110.1 ± 8.4 mmHg. Table 1 is a summary of results in which several parameters of renal function were measured in the denervated and the innervated kidney. Urine osmolality was almost same on both sides. Fractional excretion of filtered water \( (V/100 \text{ ml GFR}) \) and urine sodium excretion \( (U_{Na} / V) \) were significantly larger in the denervated kidney than in the innervated kidney (Table 1). Fractional excretion of filtered sodium \( (FE_{Na}) \) was also significantly larger on the denervated side, i.e., 0.74 ± 0.17% compared with 0.32 ± 0.12% \((\text{P} < 0.01)\).

There was no significant difference in \( C_{Cr} \) and \( C_{\text{PAH}} \) between the two sides (Table 1). Filtration fraction (FF) was the same on each side, i.e., 0.406 ± 0.058 and 0.404 ± 0.049, respectively.

Fractional sodium delivery to the distal nephron \( (C_{Na} + C_{H_{2}O}/100 \text{ ml GFR}) \) and fractional free water clearance \( (C_{H_{2}O}/100 \text{ ml GFR}) \) were significantly larger in the denervated kidney (Table 1). The relationship between \( C_{Na} + C_{H_{2}O}/100 \text{ ml GFR} \) and \( C_{H_{2}O}/100 \text{ ml GFR} \) is depicted in Fig. 1. Both parameters were closely correlated with each other and this relationship was quite similar in both the denervated and innervated kidney. Percent distal-tubule sodium reabsorption was slightly larger in the innervated kidney of all but one dog (Table 1), but the difference was not statistically significant \((0.05 < \text{P} < 0.1)\).

### DISCUSSION

We have previously reported that the denervation natriuresis and diuresis occurred without any change in GFR, RPF, and redistribution of intrarenal blood flow in both chronically and acutely denervated dogs (16, 17). In the present study the acute effects of denervation on tubular transport of water and sodium were studied using hypophysectomized, cortisone-treated, diuretic dogs. Water diuresis is difficult to develop in the anesthetized dog even after hypotonic volume expansion, and this has been attributed to excess antidiuretic hormone. Therefore, the hypophysectomy was done in order to inhibit ADH in this experiment. Under these conditions, it was clearly observed that the denervation produced a natriuresis and diuresis without associated change in GFR and RPF. This again suggests that there is a direct effect of the renal nerves on tubular transport of sodium and water. (Table 1).

Recently, Katz and Shear (15) criticized the method of the renal denervation used in our experiments, claiming that it would cause asymmetrical distribution of intrarenal blood flow and also that this might be incomplete denervation. However, we found no significant difference in the distribution of intrarenal blood flow in the denervated kidney compared to the innervated kidney as previously shown (16). Furthermore, denervation natriuresis and diuresis was clearly evident in all dogs in our experiments, suggesting that the denervation was adequate for the purpose of the study.

Under the condition of stable water diuresis in which the influence of ADH is eliminated, urine flow and solute-free water clearance are assumed to reflect delivery of glomerular filtrate out of the proximal tubule and reabsorption of sodium at the distal diluting site in the nephron (10). In addition, \( C_{Na} + C_{H_{2}O} \) is assumed to be a minimal approximation of the amount of sodium delivered to the distal nephron (19). When \( C_{Na} + C_{H_{2}O} \) is

### TABLE 1. Effect of denervation on renal function

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>( U_{Na} ), mosmol/kg H2O</th>
<th>( V / \text{ml/min per 100 ml GFR} )</th>
<th>( U_{Na} / V, \mu \text{eq/min} )</th>
<th>( C_{Na} ), ( \mu \text{eq/min} )</th>
<th>( C_{H_{2}O} ), ( \mu \text{eq/min} )</th>
<th>( U_{Na} + C_{H_{2}O}, \mu \text{eq/min per 100 ml GFR} )</th>
<th>( C_{Na} + C_{H_{2}O}, \mu \text{eq/min per 100 ml GFR} )</th>
<th>( C_{Na} + C_{H_{2}O} \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>63.51</td>
<td>8.5 6.9</td>
<td>30 8</td>
<td>20 18</td>
<td>6.7 5.7</td>
<td>7.7 5.7</td>
<td>87.0 100.0</td>
<td>140.0</td>
</tr>
<tr>
<td>15</td>
<td>62.51</td>
<td>12.8 10.3</td>
<td>33 9</td>
<td>22 20</td>
<td>10.7 8.3</td>
<td>11.8 8.7</td>
<td>90.7 95.4</td>
<td>100.0</td>
</tr>
<tr>
<td>16</td>
<td>56.70</td>
<td>7.7 5.5</td>
<td>12 1</td>
<td>35 34</td>
<td>8.1 4.0</td>
<td>6.3 4.0</td>
<td>96.8 100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>17</td>
<td>65.62</td>
<td>17.4 11.6</td>
<td>33 9</td>
<td>19 24</td>
<td>19.8 8.7</td>
<td>13.9 9.5</td>
<td>97.1 91.6</td>
<td>91.6</td>
</tr>
<tr>
<td>18</td>
<td>65.76</td>
<td>12.3 9.0</td>
<td>9 4</td>
<td>13 14</td>
<td>9.2 6.0</td>
<td>9.8 7.1</td>
<td>93.9 97.2</td>
<td>97.2</td>
</tr>
<tr>
<td>19</td>
<td>67.79</td>
<td>9.9 7.2</td>
<td>16 8</td>
<td>28 26</td>
<td>7.8 5.1</td>
<td>7.9 5.3</td>
<td>94.9 96.2</td>
<td>96.2</td>
</tr>
<tr>
<td>Mean</td>
<td>66.8 67.0</td>
<td>11.6 8.5</td>
<td>22.9 8.3</td>
<td>92.3 91.8</td>
<td>8.8 6.5</td>
<td>9.6 6.7</td>
<td>92.6 96.7</td>
<td>97.0</td>
</tr>
<tr>
<td>sE</td>
<td>4.0 4.6</td>
<td>1.1 1.6</td>
<td>2.6 2.6</td>
<td>3.2 3.2</td>
<td>1.5 1.5</td>
<td>1.7 1.9</td>
<td>1.9 1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* \( U_{Na} \), urinary osmolality; \( V \), urine flow; \( U_{Na} / V \), sodium excretion; \( V / \text{ml/min per 100 ml GFR} \), potassium excretion; \( C_{Na} \), creatinine clearance; \( U_{Na} \), p-ammoniattinate clearance; \( C_{H_{2}O} \), free water clearance; \( C_{Na} + C_{H_{2}O} \), fractional sodium reabsorption by diluting segment; \( C_{Na} \), denervated kidney; \( C_{H_{2}O} \), innervated kidney.
used in this manner, it does not take into account, at least, the fraction of sodium delivered to the distal nephron and exchanged for potassium. However, urinary potassium excretion in our study ranged from 4 to 19 μeq/min. This would contribute only 0.2-0.6 ml/min to the calculated delivery rates and would not have any significant effect on the results.

In our experiments, a moderate water load was given to hypophysectomized dogs. Although the mean plasma osmolality and plasma sodium concentration were low normal during the clearance periods, a mean urinary osmolality of less than 67 mosmol/kg H₂O suggests adequate suppression of ADH. In the denervated kidney, the delivery of glomerular filtrate out of the proximal tubule (V), the delivery of sodium to the distal nephron, and the reabsorption of sodium at distal diluting site (V₁) were all significantly greater than those of the innervated kidney. Although these results obtained by the free water clearance techniques are indirect, it is suggested that denervation inhibits sodium reabsorption in the proximal tubule and thereby increases distal sodium delivery and transport. The term distal diluting site in this context refers to the water-impermeable segment beyond which the urine is diluted, and will include the ascending limb of Henle and probably more distal tubular site.

It is well known that physical factors affect sodium and water transport in the proximal tubule. However, in this experiment RPF, GFR, and FF were same on both sides. Therefore, a denervation effect on these physical factors can be excluded.

The reabsorption of sodium at the distal diluting site was closely correlated with delivery of sodium to the distal nephron as shown in Fig. 1. Thus, an augmented reabsorption of sodium in the diluting segment in the denervated kidney can not be responsible for the change in tubular function seen with denervation. Instead, the data suggest an increase in delivery of sodium out of the proximal tubule. This is supported by the fact that there was no significant difference in percent distal tubule sodium reabsorption on both sides. Therefore, we conclude that the increase in sodium and water excretion seen with denervation results primarily from an increased rejection of glomerular filtrate in the proximal tubule. A small effect of denervation on more distal sites cannot be completely excluded.

Gill and Casper (12, 13) using the hypophysectomized and diuretic dog showed that alpha- and beta-adrenergic drugs produced changes in the proximal tubular reabsorption of sodium and water. Blendis et al. (6) could not confirm an effect of these drugs on proximal sodium reabsorption in the dog when studied by the micropuncture method.

Recently, Bencsáth et al. (3) and Bello-Reuss et al. (2) used micropuncture to study the effects of denervation on tubular function in anesthetized and non-diuretic rats. They concluded that denervation produced a marked depression of sodium reabsorption in the proximal tubule and also produced an increase of sodium reabsorption in the ascending limb and the more distal tubule and collecting ducts. The increase of sodium reabsorption in the distal nephron did not completely compensate the decrease occurring proximally.

The results of the present study, therefore, are compatible with those reported by Bencsáth et al. (3) and Bello-Reuss et al. (2) in the rat, and also indirectly support the results of Gill and Casper (12, 13) in the dog.

We are deeply indebted to Dr. Leonard B. Berman, University of California, Irvine, Calif., for his valuable help in preparing the manuscript.

Received for publication 21 April 1976.

REFERENCES


