Hepatic Denervation Chronically Elevates Arterial Pressure in Wistar-Kyoto Rats

Scott H. Carlson, John W. Osborn, J. Michael Wyss

Abstract—Several lines of evidence suggest that peripheral osmoreceptors respond to alterations in dietary NaCl by adjusting renal sympathetic nerve activity, but the impact of this reflex on the long-term regulation of mean arterial pressure (MAP) remains unclear. The present study tested the hypothesis that denervation of peripheral osmoreceptors elevates arterial pressure and induces NaCl-sensitive hypertension in normotensive rats. Hepatic denervated and sham-operated Wistar-Kyoto rats were instrumented with telemetry probes for continuous monitoring of MAP and heart rate. After 1 week on a basal (0.6%) NaCl diet, the rats were fed a high (8%) NaCl diet for 2 weeks. On the basal NaCl diet, MAP in hepatic denervated rats was 15 ± 1 mm Hg higher than in sham-operated rats. The high NaCl diet did not significantly increase MAP above baseline levels in either denervated or sham-operated rats, but the amplitude of the 24-hour rhythm of arterial pressure increased significantly more in the denervated than in the sham-operated rats. In a second experiment, two similar groups of rats were fed a very low (0.05%) NaCl diet. Mean arterial pressure of the denervated group was significantly higher than that of the sham-operated rats on either the basal or the very low NaCl diet, but the very low NaCl diet did not affect arterial pressure in either group. These results suggest that in the rat, although hepatic osmoreceptors contribute to long-term arterial pressure regulation, they contribute much less to dietary NaCl-induced changes in arterial pressure. (Hypertension. 1998;32:46-51.)

Key Words: circadian rhythm • diuresis • natriuresis • receptors • telemetry

The ability of the kidney to modify sodium reabsorption is regulated by a number of extrinsic factors, including hormonal input and neural innervation. Hormonally, the renin-angiotensin-aldosterone system, atrial natriuretic peptide, and arginine vasopressin directly modify renal sodium and water excretion. The sympathetic nervous system also regulates renal handling of sodium and water, and sympathetic activity is modified, at least in part, by increased plasma sodium and volume. As plasma sodium levels increase, water is osmotically drawn into the vascular compartment, thus increasing blood volume and thereby activating stretch-sensitive baroreceptors that modify sympathetic nervous system activity. This baroreceptor reflex is thought to be complemented by feedback from osmosensitive cells located in the hepatoporal region, which can directly detect alterations in dietary sodium and modify sympathetic nervous system activity accordingly. The concept of peripheral osmoreceptors was first introduced by Haberich in 1968 and led to early studies focused on peripheral control of vasopressin release. More recent studies demonstrate that the peripheral osmoreceptor reflex directly reduces renal sympathetic nerve activity and thus increases natriuresis and diuresis. Morita, Hosomi, and their colleagues have demonstrated that in rabbits, intraportal infusions of hypertonic saline result in an inhibition of renal sympathetic nerve activity and that hepatic denervation abolishes this sympathoinhibitory response. These authors have also reported that in dogs, ingestion of a high NaCl diet leads to an inhibition of renal nerve activity and increased natriuresis and diuresis. Both of these responses are abolished by hepatic denervation, supporting the hypothesis that peripheral osmoreceptor activity modifies sympathetic activity.

Although these data support the concept of a short-term role for the hepatic nerves in osmotic regulation, few researchers have examined the long-term role of hepatic osmoreceptors in MAP regulation. Two studies have demonstrated that disruption of the hepatic osmoreceptors (via CCl4-induced liver cirrhosis) reduces the ability of rats to excrete either dietary or infused sodium. Furthermore, Morita et al reported that hepatic denervation increased the sodium imbalance in rats fed a high (8%) NaCl diet. In these rats, MAP was significantly higher in the hepatic denervated group than in the sham-operated group. Unfortunately, MAP was not measured in a similar group of rats fed a basal NaCl diet, and thus it remains unclear whether the observed increase in arterial pressure resulted from the denervation, the high NaCl diet, or both. Although these aforementioned studies suggest a long-term role for the hepatic nerves in NaCl-sensitive blood pressure regulation, they do not directly test this hypothesis. Therefore, the present study employed telemetric techniques, which facilitate precise and continuous moni-
toring of arterial pressure and HR in freely moving, untethered animals. With this technique, the experiments tested the hypothesis that hepatic denervation increases arterial pressure and induces dietary NaCl-sensitive increases in arterial pressure in normotensive rats.

Methods

Nine-week-old male WKY (Taconic Farms, Germantown, NY) were used in all experiments. All rats were housed in individual cages in a sound-attenuated room at constant humidity (60±5%), temperature (24±1°C), and light cycle (6 AM to 6 PM). All rats were fed ad libitum access to an initial basal NaCl diet (0.6%; diet No. 8746, Teklad) and water. All studies were conducted in accordance with institutional and National Institutes of Health guidelines for experiments with animals.

Surgical Procedures

Rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg body wt), and the portal vein was exposed via a midline abdominal incision. The hepatic nerves were transected as described previously. In brief, the portal vein was isolated and the segment immediately adjacent to the liver was stripped of all surrounding tissue. This process was then repeated on the hepatic artery and bile duct so that there were no visible strands of tissue connecting the vessels to the liver. The area was then swabbed with a 10% phenol–ethanol solution. The sham surgery was identical except that no tissue was cleared nor was the 10% phenol–ethanol solution applied. After the hepatic surgery, a segment of the aorta was cleared just below the renal arteries, and the flexible tip of the telemetry wire was surgically sutured into the abdominal wall, after which the incision was closed. The rat was then returned to its home cage and allowed to recover for 3 days before the start of the experiment.

Experimental Protocol

After surgery, the denervated (n=7) and sham-operated (n=6) rats were maintained on a basal NaCl diet (0.6%; diet No. 8746) for at least 1 week until MAP had stabilized for at least 4 consecutive days. Rats were then fed a high NaCl diet (8%, diet No. 5008) for 2 weeks, after which they were returned to the basal NaCl diet for 3 days.

To address the issue of salt sensitivity variability, experiment 1 was repeated in a second group of denervated (n=5) and sham-operated (n=3) animals that were maintained on a basal NaCl diet for 1 week and then switched to a very low NaCl (0.05%; diet No. 5016) diet for 1 week.

Data Acquisition and Analysis

Continuous 24-hour MAP, HR, and activity were monitored in unrestricted and untethered animals with the use of the Dataquest IV system (Data Sciences Inc), which consists of the implanted radiofrequency transmitter and the receiver, which was placed under each cage. The output was relayed from the receiver through a consolidation matrix to a personal computer.

Individual 10-second waveforms of MAP, HR, and activity were sampled every 5 minutes throughout the course of the study, and hourly averages and SDs were then calculated. Individual 24-, 12-, and 6-hour means and daily peak and nadir values were calculated from the individual hourly averages and analyzed for intragroup and between-group comparisons.

Circadian rhythm analysis of the individual hourly MAP and HR data was performed with the nonlinear, least-squares fitting program PHARMFIT, and the “best-fit” model was defined as the one with the lowest number of harmonics that had a confidence value of at least 0.05, as determined by the subprogram SYNOPS. All PHARMFIT analyses were based on data for 3 consecutive days, thus allowing comparisons of the harmonic patterns in each group and the mean MESOR, amplitude, and acrophase (clock time of peak amplitude) of the 24-hour adjusted rhythm.

All data were evaluated by ANOVA (significance criteria of P<0.05) with appropriate post hoc tests (Newman-Keuls) to determine the source of main effects and interactions.

Verification of Denervation
After completion of the experiments, the rats were deeply anesthetized with urethane, and a section of liver was removed, immediately frozen in LN₂, and stored at −80°C. The tissue was later extracted for measurement of tissue NE levels as described elsewhere. In brief, the tissue was homogenized in a 0.1 mol/L HClO₄–EDTA solution and centrifuged, and a portion of the supernatant was extracted with the use of acid-washed alumina in 1.5 mol/L Tris and 0.1 mol/L phosphate buffer. The samples were then analyzed for NE by high-performance liquid chromatography with electrochemical detection (HPLC-EC, Waters Corp), and the values were adjusted for recovery with the use of 3,4-dihydrobenzylamine as an internal standard. As observed previously, hepatic denervation significantly decreases liver NE concentration. Individual rats were excluded from the present study if their hepatic NE levels were >10% of those in sham-operated rats. A total of two rats were excluded from the denervated group on this basis.

Results

Experiment 1

Hepatic denervation increased MAP in WKY maintained on either the basal (0.6%) or high (8%) NaCl diet (Figures 1 and 2). The 24-hour MAP (MESOR) was significantly elevated (15±1 mm Hg. P<0.05) in the denervated rats compared with the sham-operated group maintained on the basal diet (Figures 1 and 2 and Table 1). Both intact and denervated WKY displayed a 24-hour circadian rhythm with similar amplitude, acrophase, and goodness of fit during the control period on the basal NaCl diet (Table 1). After exposure to the high NaCl diet, MAP remained significantly higher in the denervated than in the sham-operated rats (Figures 1 and 2 and Table 1), but the high NaCl diet did not significantly elevate MAP above control levels in either group (Figure 2 and Table 1). The high NaCl diet caused a greater absolute and percent increase in the amplitude of the 24-hour MAP rhythm in the denervated compared with the sham-operated rats (150±4% versus 83±8%, respectively, P<0.05; Table 1). When both groups were returned to the basal NaCl diet, MAP in the denervated group decreased slightly below its initial baseline, whereas MAP in the sham-operated group returned to its initial baseline (Figure 2 and Table 1).

Further analysis demonstrated that during the 12-hour light/dark segments and during the 6-hour time segments, the high NaCl diet significantly elevated MAP above control levels in both groups during the second nighttime period (12 AM to 6 AM; data not shown). The daily peak and nadir MAP rhythms were calculated from the individual hourly averages (Figure 3). The 24-hour MAP peak was significantly higher in the denervated group than in the sham-operated group throughout the entire study, but the nadir in MAP was

**Selected Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>MESOR</td>
<td>midline estimating statistic of rhythm</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto rats</td>
</tr>
</tbody>
</table>
elevated only during the initial control period and on a few days during the high NaCl diet (Figure 3).

Throughout the study there was no significant difference between the groups in any component of the 24-hour HR rhythm (Table 2). In both groups, exposure to the high NaCl diet initially tended to increase HR, after which HR declined to levels significantly lower than their basal NaCl diet baseline (data not shown).

**Experiment 2**

In the second study, the difference in basal MAP between the denervated and sham-operated groups was approximately the same as that observed in experiment 1. MAP in the denervated group was significantly higher (13 ± 6 mm Hg) than in the sham-operated rats (Figure 4 and Table 3). Maintenance on the very low NaCl (0.05%) diet tended to decrease MAP of the denervated rats for the duration of the study; however, this decline did not reach statistical significance. During maintenance on the very low NaCl diet, MAP of the denervated group remained significantly elevated above sham levels (Figure 4 and Table 3). Furthermore, the amplitude and acrophase of the MAP rhythm were similar between groups and did not change significantly after exposure to the very low NaCl diet (Table 3).

**Discussion**

The kidney plays an important role in regulating sodium and water balance, and sympathetic nervous system innervation is known to modify this function. Although renal sympathetic nerve activity is regulated by baroreceptor feedback and central nervous system control, recent evidence suggests that the hepatic nerves also influence renal sympathetic nerve

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**TABLE 1. 24-Hour MAP Rhythms in Denervated (n=7) and Sham-Operated (n=6) WKY Maintained on 0.6% and 8% NaCl Diets**

<table>
<thead>
<tr>
<th>Group</th>
<th>MESOR, mm Hg</th>
<th>Amplitude, mm Hg</th>
<th>Acrophase, 24-Hour Clock</th>
<th>Rhythm, % Best Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham, control</td>
<td>95±0.4</td>
<td>6.4±0.5</td>
<td>23:21±0.6</td>
<td>62</td>
</tr>
<tr>
<td>Denervated, control</td>
<td>109±0.4*</td>
<td>6.1±0.5</td>
<td>23:15±0.7</td>
<td>62</td>
</tr>
<tr>
<td>Sham, 8%: days 3–5</td>
<td>101±0.5</td>
<td>10.1±0.7</td>
<td>0:53±0.5</td>
<td>75</td>
</tr>
<tr>
<td>Denervated, 8%: days 3–5</td>
<td>111±0.3*</td>
<td>11.7±0.5</td>
<td>2:00±0.4</td>
<td>79</td>
</tr>
<tr>
<td>Sham, 8%: days 12–14</td>
<td>100±0.5</td>
<td>11.7±0.7</td>
<td>0:52±0.5</td>
<td>78</td>
</tr>
<tr>
<td>Denervated, 8%: days 12–14</td>
<td>108±0.5*</td>
<td>15.1±0.6*</td>
<td>1:32±0.3</td>
<td>78</td>
</tr>
<tr>
<td>Sham, recovery</td>
<td>95±0.5</td>
<td>8.2±0.7</td>
<td>0:24±0.6</td>
<td>59</td>
</tr>
<tr>
<td>Denervated, recovery</td>
<td>103±0.4†</td>
<td>6.9±0.6</td>
<td>0:34±0.6</td>
<td>59</td>
</tr>
</tbody>
</table>

*P<0.05 vs sham group on the same diet.
†P<0.05 vs the control diet.
activity in response to the ingestion of NaCl. \textsuperscript{10–12,14} This response of the hepatic nerves to a change in dietary NaCl is thought to be mediated by osmosensitive cells (peripheral osmoreceptors) in the liver. The majority of studies addressing hepatic osmoreceptor feedback have examined responses of only short-term peripheral osmoreceptor activation, and therefore the overall significance of these receptors in long-term regulation of MAP is unknown. The present study examined the effect of hepatic denervation on the ability of the rat to maintain a normal MAP when challenged with a high NaCl diet. The major findings indicate that hepatic denervation produces a consistent rise in arterial pressure in rats maintained on a normal sodium diet but that denervation has little effect on the arterial pressure response to alterations in dietary NaCl, at least in WKY.

We hypothesize that over the long term, hepatic denervation elevates arterial pressure through disruption of the rat’s normal sodium and water balance, although neither sodium nor water balance was measured in this study. Such a proposal is supported by the work of Lopez-Novoa and Martinez-Maldonado,\textsuperscript{16} who demonstrated that in rats, intra-splenic (compared with intravenous) infusion of hypertonic saline elicited greater sodium and chloride excretion. Furthermore, CCl\textsubscript{4}-induced liver cirrhosis (a model for hepatic denervation) greatly impaired the natriuretic response to the intrasplenic infusion, suggesting that hepatoportal dysfunction results in a sodium imbalance.

Similarly, Tanaka et al\textsuperscript{15} used the cirrhotic rat model to examine the long-term effect of the hepatic nerves on long-term sodium balance in rats fed either a basal NaCl diet or a high NaCl diet. Although the induction of cirrhosis had no effect on overall sodium balance when the rats were maintained on the basal NaCl diet, cirrhotic rats exposed to the high NaCl diet retained significantly more sodium than did control rats. Furthermore, the cirrhotic rats displayed significantly reduced hepatic nerve responses to intraportal hypertonic saline infusion. In a subsequent study,\textsuperscript{17} the effect of hepatic denervation on long-term sodium balance was measured in rats initially

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### Table 2. 24-Hour HR Rhythms in Denervated (n=7) and Sham-Operated (n=6) WKY Maintained on 0.6% and 8% NaCl Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>MESOR, bpm</th>
<th>Amplitude, bpm</th>
<th>Acrophase, 24-Hour Clock</th>
<th>Rhythm, % Best Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham, control</td>
<td>358±1.2</td>
<td>75±1.6</td>
<td>22:55±0.2</td>
<td>82</td>
</tr>
<tr>
<td>Denervated, control</td>
<td>365±1.3</td>
<td>68±1.8</td>
<td>23:15±0.2</td>
<td>83</td>
</tr>
<tr>
<td>Sham, 8%: days 3–5</td>
<td>373±0.5</td>
<td>79±0.8</td>
<td>0:09±0.5</td>
<td>87</td>
</tr>
<tr>
<td>Denervated, 8%: days 3–5</td>
<td>373±1.5</td>
<td>68±2.1</td>
<td>0:00±0.2</td>
<td>90</td>
</tr>
<tr>
<td>Sham, 8%: days 12–14</td>
<td>351±1.4</td>
<td>80±1.8</td>
<td>0:01±0.2</td>
<td>87</td>
</tr>
<tr>
<td>Denervated, 8%: days 12–14</td>
<td>340±1.3</td>
<td>75±1.6</td>
<td>23:52±0.2</td>
<td>92</td>
</tr>
<tr>
<td>Sham, recovery</td>
<td>345±1.4</td>
<td>77±1.9</td>
<td>23:38±0.2</td>
<td>90</td>
</tr>
<tr>
<td>Denervated, recovery</td>
<td>344±1.2</td>
<td>76±1.7</td>
<td>23:26±0.2</td>
<td>91</td>
</tr>
</tbody>
</table>
maintained on a 0.45% NaCl diet followed by a high NaCl diet. Tail-cuff measurements were used to determine the systolic blood pressure during the study, and at the conclusion of the experiments, direct arterial pressure was taken, but only in rats on an 8% NaCl diet. Although hepatic denervation had no effect on either sodium balance or systolic arterial pressure in rats fed a 0.45% NaCl diet, in the rats fed the 8% NaCl diet, denervation (compared with sham operation) increased sodium balance to a greater extent. Furthermore, the MAP of denervated rats fed an 8% NaCl diet was ≈12 mm Hg higher than that of sham-operated rats. Interestingly, the magnitude of this elevation of MAP is similar to that observed in rats on the basal NaCl diet in the present study. Because Morita et al.17 did not measure MAP in denervated rats fed the basal NaCl diet, it is possible that MAP was also elevated in their rats even on the basal NaCl diet.

If hepatic denervation elevates arterial pressure by disrupting sodium and water homeostasis, then exposure to the high NaCl diet should have exacerbated sodium and volume retention, thereby further increasing arterial pressure. The present study demonstrated that neither the high NaCl diet nor the very low NaCl diet markedly altered arterial pressure. However, hepatic denervation still appears to play a small role in long-term arterial pressure responses to sodium intake in the rat. The high NaCl diet increased the amplitude of the 24-hour MAP rhythm more in the denervated than in the sham-operated rats. This observation suggests that hepatic denervation lengthens the time needed for rats to clear the volume and sodium load after ingestion. Verification of this hypothesis will require metabolic studies.

Although the hepatic nerves may assist in regulating the rat’s sodium and water balance, the mechanism by which this homeostasis is accomplished is unclear. Osmosensitive cells located in the hepatoporal region are sensitive to ingested sodium, and these peripheral osmoreceptors appear to contribute to control of renal sympathetic nerve activity.10–12 Thus, the peripheral osmoreceptors may serve to modulate renal handling of sodium. However, peripheral osmoreceptors also appear to control the release of vasopressin,2,22 drinking behavior,13–25 and gastrointestinal absorption of sodium and water.26,27 Thus, the effects of hepatic denervation are likely multifaceted.

There are several other mechanisms by which hepatic denervation may have affected arterial pressure in the present study. Removal of efferent control of the liver could have raised arterial pressure, or disruption of the innervation of the hepatic artery and portal vein may have resulted in an inappropriate vasoconstriction of these vessels, thereby increasing total peripheral resistance. It is also possible that the putative hepatic baroreceptors28,29 exert tonic inhibition on sympathetic activity and that hepatic denervation removes this afferent input. Conversely, it is unlikely that the denervation process significantly compromised the health of the rats. The denervated rats were healthy and active throughout the study and displayed a normal circadian rhythm for arterial pressure, HR, and activity. We cannot discount the possibility that application of the 10% phenol–ethanol solution may have directly affected the liver or other tissue (thereby elevating arterial pressure), because the sham-operated rats were not exposed to the phenol application; however, such a possibility seems unlikely. This technique has been used extensively in previous studies, and no signs of damage to the liver or other organs have been reported.10,11 In contrast to earlier experiments30 in which the 10% phenol solution was simply poured onto the hepatic artery and portal vein, in the present study a 10% phenol solution was painted only on the blood vessels of interest, thereby reducing spread of the solution and minimizing any potential effects on the surrounding tissue. Furthermore, we have previously used the phenol-ethanol mixture to denervate the kidney (a site remote from the liver) and have found that the effects of this denervation are kidney specific and do not mimic those in the present report.30 Specifically, renal denervation decreased arterial pressure but did not affect salt-sensitive hypertension.30

It will be important in future studies to examine the effects of hepatic denervation on experimental models that display compromised sodium or arterial pressure regulation. WKY may have enough compensatory or redundant mechanisms to mask the effects of hepatic denervation on NaCl-sensitive hypertension. Future studies should focus on the significance of the peripheral osmoreceptor reflex in other rat models, like the spontaneously hypertensive rat, which displays salt-sensitive forms of hypertension. The spontaneously hypertensive rat lacks numerous compensatory mechanisms for maintaining normal arterial pressure, and the loss of peripheral osmoreceptors may be more critical in this model.

In summary, this is the first study to employ telemetric, circadian rhythm analysis to test the contribution of peripheral osmoreceptors to chronic MAP regulation. The results demonstrate that in WKY, hepatic denervation causes long-term elevation in MAP but does not appear to appreciably increase arterial pressure responses to changes in dietary NaCl.

### Acknowledgments

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