Altered Neural Control of Cardiovascular Function in Sodium-Depleted Dogs

SHUICHI TAKISHITA, M.D., AND CARLOS M. FERRARIO, M.D.

SUMMARY Sodium restriction affects sympathetic control mechanisms by blunting of the reflex pressor response to carotid sinus hypotension, which is reversible by section of vagal afferents. To obtain more direct evidence, sympathetic nerve activity was recorded from a renal nerve (RNA) in 16 normal (NS) and 13 sodium-depleted (SD) dogs anesthetized with morphine-pentobarbital. Integrated RNA was measured during changes in mean arterial pressure (MAP) produced by i.v. infusion of sodium nitroprusside (100 μg/kg/min) or phenylephrine (20 μg/kg/min). The classical inverse relationship between MAP and integrated RNA was found before and after bilateral vagotomy (VAGT) in both NS and SD dogs. However, RNA in SD dogs, expressed as % of maximal neural firing, was significantly less at any blood pressure level when compared to NS dogs. In addition, the critical pressure (point at which RNA ceased) was reduced in SD vs NS dogs (p < 0.002). The decreased sympathetic neural firing in SD dogs was abolished after bilateral VAGT, confirming the pronounced buffering effects of vagal afferents on RNA in salt-depleted dogs.

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KEY WORDS • sodium depletion • renal sympathetic nerve activity • blood pressure • cardiopulmonary receptors • renin-angiotensin system • hypertension

CHRONIC sodium depletion (SD) may produce significant alterations in autonomic nervous system function that do not conform to the usual understanding of the effect of increased blood levels of angiotensin II (All) upon neurogenic drive. Although All certainly acts to augment sympathetic efferent activity, in the dog the state of the sodium balance appears to modify significantly, and in an unexpected manner, the expression of the interplay between the renal pressor and the sympathetic nervous systems.

A series of correlated studies involving investigation of baroreceptor reflexes, cardiac function, and measurements of catecholamines in both plasma and cerebrospinal fluid support this conclusion. The studies were prompted when the carotid occlusion (CO) reflex was found to be sharply inhibited in dogs subjected to SD of 3 weeks' duration. Suppression of the characteristic rises in heart rate and cardiac output persisted even after cholinergic blockade with atropine. However, section of the cervical vagus nerves markedly potentiated the CO reflex to the levels found in normal vagotomized animals. The augmented pressor response was now accompanied by prominent increases in stroke volume and cardiac output. These data suggested that, in the SD state, removal of the inhibitory activity from the carotid sinus baroreceptors during CO was not sufficient to liberate the vasomotor centers from the additional input of vagal afferents probably discharging at rates above their tonic normal influence.

The objective of the present study was to determine whether this peculiar effect of SD upon cardiovascular reflexes could be documented more directly by electrophysiological recordings of sympathetic nerve activity both before and after section of the vagus nerves.

Methods

Experiments were performed in 29 male mongrel dogs weighing between 17 and 23 kg. Thirteen animals were fed a sodium-restricted diet containing < 4 mEq Na+/day (Prescription Diet, h/d, W.A. Butler, Warren, Ohio) for 21 days. Sixteen other dogs ate a control diet containing 65 mEq Na+/day (Purina Dog Chow, Fetzer Brothers, Bedford, Ohio). To enhance the degree of SD, dogs were given furosemide (40 mg i.m., Lasix, Hoechst-Roussel Pharmaceuticals, Somerville, New Jersey) on the first and last 2 days of the diet. The degree of sodium deficiency was gauged from changes in plasma renin activity (PRA, measured by radioimmunoassay) as well as concurrent alterations in hematocrit, serum proteins, plasma electrolytes, blood urea nitrogen, and serum creatinine. These assays were performed as described previously with blood collected by venipuncture on the first and last days of the dietary regime.

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Animal Preparation

Dogs were anesthetized with sodium pentobarbital (15 mg/kg body weight, i.v.) after premedication with morphine (2 mg/kg, i.m.) and mechanically ventilated. Arterial pressure (AP) was recorded in the abdominal aorta from a cannulated femoral artery; heart rate (HR) was monitored using a biotachometer triggered by the R-R interval of the simultaneously obtained electrocardiogram (ECG). Test drugs were infused via cannulated femoral veins. Loose silk ligatures were placed around both cervical vagus sympathetic trunks. The surgical procedures were completed by exposing the renal sympathetic nerve plexus through a retroperitoneal incision in the left flank. Under an operating microscope, a renal nerve was dissected free from the surface of the renal vascular plexus, cut peripherally, and the central end freed of connective tissue and sheath. After placement on platinum wire electrodes, the area was flooded with mineral oil to prevent drying of the tissues. In separate experiments we have established that these nerves carry postganglionic nerve activity since the nerve discharges ceased after ganglionic blockade with hexamethonium.

Efferent activity in the postganglionic renal nerves was measured electroneurographically and correlated with blood pressure responses to the intravenous administration of vasoactive drugs. Sodium nitroprusside (100 μg/kg; Nipride, Roche Laboratories, Nutley, New Jersey) and phenylephrine hydrochloride (20 μg/kg; Neo-Synephrine, Winthrop Laboratories, New York, New York) were infused systemically via separate catheters at a rate of 1.23 ml/min. Two different tests were performed, the first consisting of sequential infusions of nitroprusside and phenylephrine to produce continuous changes in mean arterial pressure (MAP) ranging from about 40 mm Hg below to 60 mm Hg above preinfusion levels. The second test was to infuse phenylephrine alone in an amount sufficient to raise the AP by at least 60 mm Hg from baseline values. Changes in basal levels of AP and HR due to the action of the vasoactive drugs were avoided by waiting 40 to 50 minutes between the first and second infusion periods. These tests were repeated following section of both cervical vagus nerves in all 13 SD dogs and in eight of the 16 normal animals. All experiments were completed by recording the residual electrical activity present 15 minutes after a lethal dose of intravenous pentobarbital.

Recording Techniques

Details of the procedures as used are described elsewhere. Briefly, electrical activity from a renal nerve was obtained with a high gain capacitance coupled amplifier (Model 113 AC/DC differential amplifier, Princeton Applied Research, Princeton, New Jersey) at a bandwidth frequency between 100 and 3000 Hz. The amplifier's output was displayed on the face of a cathode ray tube and photographed on moving paper together with the AP and the ECG. The electroneurogram signal was also fed in parallel to an absolute value amplifier coupled to an RC integrating circuit with a 1-second time constant. The output of the integrator was reset to baseline levels every 4.5 seconds; therefore, the nerve output data are denoted as the mean integrated renal nerve activity (RNA) over this time period. A DC voltage applied to the output of the absolute gain amplifier prevented the addition of 60 Hz noise to the integration. Additional corrections were obtained by determining the absolute zero electrical activity 15 minutes after euthanasia and subtracting this value from the integration. Variables were simultaneously displayed on an ink writing polygraph and also stored on FM tapes. All values are reported as means ± SEM. Statistical evaluation was made by Student's t test for either paired or unpaired observations. Correlations were calculated by a linear least-squares fit. A value of p < 0.05 was considered significant.

Results

Dogs subjected to a restricted sodium intake and diuretic therapy showed decreased body weight, hemococoncentration, hyperproteinemia, and hyperreninemia (table 1). These data agree with those reported previously. After anesthesia but before manipulation of the renal nerve plexus, MAP averaged 96 ± 2 mm Hg and HR 67 ± 6 beats/min in 13 SD dogs. Corresponding averages in 16 normal (NS) dogs were 97 ± 3 mm Hg and 57 ± 4 beats/min. These differences were not statistically significant (p > 0.1).

Table 2 shows the grouped values of MAP and HR prior to each manipulation of blood pressure. Repeated infusions of the vasoactive agents did not have a persistent effect on AP and HR. On each occasion, similar baseline values were reached before administration of the next infusion whether comparisons were made within the groups or across them. Section of the vagus nerves caused tachycardia and an elevation of MAP of about equal magnitude in NS and SD dogs (table 2). There was, however, one excep-

<table>
<thead>
<tr>
<th>Table 1. Humoral Changes due to Restricted Sodium Intake and Diuretic Therapy</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
</tr>
<tr>
<td>Serum protein (g/dl)</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
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<tr>
<td>Serum creatinine (mg/dl)</td>
</tr>
<tr>
<td>Serum sodium (mEq/liter)</td>
</tr>
<tr>
<td>Serum potassium (mEq/liter)</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
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</tbody>
</table>

Values are means ± 1 SE of measurements taken in 13 conscious dogs before (control) and at the completion of the treatment regime (3rd week).

*p < 0.05.

†p < 0.001.
TABLE 2. Baseline Values of Mean Arterial Pressure and Heart Rate Preceding Each Test Procedure

<table>
<thead>
<tr>
<th></th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NS SD</td>
<td>NS SD</td>
</tr>
<tr>
<td>Before bilateral vagotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before N-P</td>
<td>97 ± 3 101 ± 2</td>
<td>72 ± 7 88 ± 6</td>
</tr>
<tr>
<td>Before P</td>
<td>94 ± 3 100 ± 3</td>
<td>68 ± 7 81 ± 5</td>
</tr>
<tr>
<td>After bilateral vagotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before N-P</td>
<td>106 ± 5 111 ± 3</td>
<td>137 ± 12 143 ± 5</td>
</tr>
<tr>
<td>Before P</td>
<td>102 ± 3 112 ± 3*</td>
<td>127 ± 14 137 ± 5</td>
</tr>
</tbody>
</table>

N-P = infusion of nitroprusside followed by phenylephrine; P = single infusion of phenylephrine; NS = normal sodium group; and SD = sodium depleted group.

*p < 0.05 compared to corresponding value in NS dogs.

...tion. Compared to values in the NS group, the preinfusion values of MAP but not HR were higher in the SD group following vagotomy and before the last phenylephrine test (table 2).

To critically assess the effects of these drugs upon AP and renal sympathetic activity, we also measured: 1) the reflex response of HR as a function of the change in MAP; and 2) the time taken for the AP to change by a predetermined increment of ± 10 mm Hg during infusion of each drug. These data are shown in figures 1 and 2 respectively. With regard to the reflex change in HR during both drug-induced hypotension and hypertension, these were within the same range in both groups (fig. 1). On the other hand, the rate at which MAP changed by a determined increment of ± 10 mm Hg during the infusion of either nitroprusside or phenylephrine was not the same in NS compared to SD dogs (fig. 2). In the experimental group, a longer time (i.e., a greater amount of drug) was needed to achieve a change in AP equal to that recorded in NS dogs. Section of the vagus nerves shortened the time needed to reach a new level of AP; however, differences between NS and SD dogs were still evident, particularly during infusion of phenylephrine (fig. 2).

Figure 3 shows tracings of phasic renal sympathetic nerve activity (RNA) along with AP and the ECG before and during the sequential infusion of nitroprusside and phenylephrine. The residual electroneuogram 15 minutes after euthanasia is also included. As shown in figure 3, normal RNA prior to the administration of the drugs was pulsative. Total activity increased sharply during the fall in AP produced by nitroprusside, whereas infusion of phenylephrine caused complete disappearance of RNA. In this and all other dogs, the residual voltage at the summit of the pressor response was identical to that recorded 15 minutes after cessation of cardiac and respiratory activity.

Figure 4 shows the relationship between integrated RNA and the change in MAP from baseline values. The values of nerve activity on the ordinate are expressed as percent of the maximum static outflow of sympathetic discharges when the MAP was lowered to 57 ± 3 mm Hg in NS and 63 ± 2 mm Hg in SD dogs.

**Figure 1.** Reflex response of heart rate to changes in mean arterial pressure (MAP) is essentially the same in 16 normal (black circles) and 13 sodium-depleted (open circles) dogs. Control values are shown in table 2.
This difference is not statistically significant \((p > 0.1)\). In 16 NS dogs, integrated RNA was consistently greater than that recorded in 13 SD dogs at each calculated level of MAP. This is illustrated by the shift to the left of the function curve in SD dogs. Moreover, the point at which RNA ceased with respect to baseline MAP (critical pressure) averaged +14 ± 3 mm Hg in 16 NS compared with +1 ± 2 mm Hg in 13 SD dogs \((p < 0.02)\). In eight of the 16 NS dogs, the relationship between RNA and MAP was obtained again after section of both cervical vagus nerves. Vagotomy was without effect. In addition, the critical pressure remained unaffected \((+16 ± 5 \text{ vs } +12 ± 3 \text{ mm Hg before vagotomy}, \ p > 0.1)\). On the other hand, the converse was true in 13 SD dogs. Following section of the vagus nerves there was a marked increase in the percent change of integrated RNA at any level of MAP, with the points now overlapping those measured in the normal group (fig. 4). The critical pressure, which before section of the vagus nerves averaged +1 ± 2 mm Hg, increased to +16 ± 3 mm Hg after vagotomy \((p < 0.01)\), a value now not different from that recorded in the normal group \((p > 0.1)\).

The average slopes of the stimulus response relation between changes in MAP and RNA for both NS and SD dogs are shown in table 3. Both the slope and the intercept of the y-axis, calculated when \(x = 0\), were statistically different between the two groups before but not after bilateral section of the vagus nerves. The latter procedure produced a significant change in both the intercept and the slope \((-2.64 ± 0.03 \text{ before vs } -2.19 ± 0.02 \text{ after}; \ p < 0.05\), table 3) of the SD group. No changes were observed in normal animals (table 3).

The critical pressure was also measured during the separate infusion of phenylephrine to ascertain that a prior hypotensive period did not affect the measurements of this variable unduly. In the main, section of the vagus nerves caused a 92\% increase in the critical pressure of SD dogs \((p < 0.02)\). In NS dogs, it increased by 36\% but this change was not statistically significant \((p > 0.1)\). Correlations of the critical pressure with baseline MAP and HR in NS were significant, with the former \((r = 0.57, \ p < 0.05)\) but not with the latter \((r = 0.34, \ p > 0.1)\). On the other hand, there was no correlation between critical pressure and baseline MAP in SD dogs \((r = 0.40, \ p > 0.1)\).

### TABLE 3. Correlations Between Changes in Mean Arterial Pressure and Integrated Renal Nerve Activity in Normal and Sodium Depleted Dogs

<table>
<thead>
<tr>
<th></th>
<th>No. of dogs</th>
<th>Intercept (% RNA)</th>
<th>Slope (% RNA/mm Hg)</th>
<th>(r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vagotomy</td>
<td>NS</td>
<td>16</td>
<td>31 ± 0.2</td>
<td>-2.22 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>8</td>
<td>29 ± 0.4</td>
<td>-2.27 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>13</td>
<td>7 ± 0.5*</td>
<td>-2.64 ± 0.03*</td>
</tr>
<tr>
<td>After vagotomy</td>
<td>NS</td>
<td>8</td>
<td>24 ± 0.5</td>
<td>-2.23 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>13</td>
<td>26 ± 0.2</td>
<td>-2.19 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± 1 SE of correlation parameters in normal (NS) and sodium depleted (SD) dogs.

\*\(p < 0.05\) for SD vs NS before vagotomy and SD after vagotomy. All other comparisons were not statistically significant.
In each film strip, the top tracing is the dog’s electrocardiogram, the middle tracing is the arterial pressure, and the lower tracing is the electrical activity in a left renal nerve of an NS dog. The pulsatile activity (top-most strip) becomes essentially continuous during the fall in blood pressure produced by nitroprusside (third strip down) and then ceases during the hypertensive response to the infusion of phenylephrine (fourth and fifth strips down). Following a lethal dose of pentobarbital (lowermost strip), the residual electrical noise is not different from that recorded at the peak of the pressor response (fifth strip down).
**Discussion**

Reduced intake of sodium chloride produces significant alterations in the reflex control of cardiovascular function in anesthetized dogs. The experiments extend previous observations of a blunted carotid sinus baroreceptor reflex in SD dogs by demonstrating electroneurographically that the discharge of renal sympathetic nerves during change in MAP is proportionally less in SD dogs. The inhibition appears to result from an increased activity of cardiopulmonary afferents because section of the cervical vagus nerves abolished previous differences in both the slope of the MAP-RNA function curve and the critical pressure between the two groups. These results are consistent with other studies which showed that in SD dogs the decreased pressor response to carotid occlusion was reversed by cervical vagotomy but not after cholinergic blockade with atropine. We have also shown that the aortic depressor nerves do not account for the reduction in sympathetic activity observed in SD dogs. Thus, the effects of vagotomy upon RNA may reflect removal of increased afferent activity. The alternate possibility of a central resetting of the interaction between low and high pressure baroreceptors cannot be excluded.

Measurements of sympathetic nerve traffic as a function of MAP provide a quantitative estimate of the regulation of AP by the baroreceptor reflexes. Within a physiological pressure range, an inverse relationship has been demonstrated between sympathetic nerve activity and carotid sinus pressure. This relationship is characterized by both the slope of the function curve as well as the point at which nerve discharges are suppressed to noise levels (critical pressure). The multifiber electroneurograms obtained in our experiments confirm that sympathetic discharges in the renal nerve of anesthetized dogs display rhythmicity with the heart beat and are also modified by respiration. As performed previously by others, the integrated nerve data were normalized from the point of highest nerve traffic obtained when the MAP was approximately at or below the known threshold for the baroreceptor reflexes. The procedure of removing from the output of the integrator the electrical noise remaining after cessation of all respiratory and cardiac activities was an added precaution to ensure accurate results.

As observed previously, SD did not affect baseline MAP and HR. Similarly, the magnitude of the reflex tachycardia and bradycardia associated with the infusion of either nitroprusside or phenylephrine respectively was essentially the same in both groups. On the other hand, MAP in the SD dogs during infusion of the drugs changed at a rate slower than that recorded in the NS group. In other words, greater quantities of the drugs were needed to cause an equivalent change in MAP. The findings are inter-
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preted to reflect known alterations in vascular resistance and reactivity accompanying sodium depletion. Several investigators have shown that after sodium depletion blood pressure is maintained by the renin-angiotensin system. Characteristically, vascular resistance in SD dogs is increased several fold whereas cardiac output is reduced proportionally. The partial recovery in the vascular reactivity of SD dogs after section of the vagus nerves is assumed to depend in part, upon a betterment of circulatory dynamics based on observations of a marked improvement in ventricular function after bilateral vagotomy in SD dogs. While other factors could also play a role, it is unlikely that the reduced sensitivity of the vascular system of SD dogs to the vasoactive drugs accounts for findings of reduced RNA. Following section of the vagus nerves, integrated RNA was restored to normal even though the animals continued to show a reduced peripheral vascular responsiveness (fig. 2). Moreover, there was no correlation between the critical pressure and the rapidity of change in MAP. From the data reported by Ninomiya et al., we deduced that a more gradual rise in MAP would be associated with a higher value of the critical pressure. However, the opposite occurred in sodium-depleted dogs.

In all experiments RNA was inversely related to MAP. However, in SD dogs the stimulus response curve was positioned to the left of that recorded in NS animals, and the critical pressure was significantly less. On the average, the slope of the stimulus response curve relating changes in pressure and RNA before vagotomy was significantly steeper in 13 SD dogs compared to the 16 NS animals. Following section of the vagus nerves, the slope in the SD group decreased significantly to a value that was not different from that observed in the NS dogs both before and after vagotomy. We therefore conclude that in the presence of the vagus nerves there are important quantitative differences in the MAP-RNA relationship between NS and SD dogs.

In recent years, data indicate that cardiopulmonary receptors influence efferent sympathetic discharges to the heart and blood vessels. Cardiopulmonary baroreceptor control of renal function not only influences the regulation of extracellular fluid volume but also appears to modulate efferent RNA. In NS dogs, vagotomy did not affect the slope of the relation between MAP and RNA. Although the critical pressure did increase after vagotomy, the change was not statistically significant. While we do not dispute that cardiopulmonary afferents influence cardiovascular function, in both the present and a previous study we were unable to observe a marked modulatory effect of these low pressure receptors upon the carotid sinus reflex of NS dogs.

There is reason to believe that the increased inhibitory influence of vagal afferents upon renal sympathetic outflow in SD dogs reflects alterations in the cardiopulmonary region. We have shown that the fraction of the blood volume contained in the cardio-pulmonary region is proportionally greater in SD animals and that hypovolemia is present while central venous pressure is unchanged. Moreover, ejection fraction was reduced markedly in SD dogs. When assessed collectively, these findings provide insight into the mechanism for the reduced response of renal nerve discharges in SD dogs. If sodium depletion is associated with reduced cardiac function, increased stretch of cardiopulmonary receptors may limit the renal sympathetic response to acute variations in arterial pressure.

It is not known whether the altered reflex responsiveness of the renal sympathetic nerves to acute variations in pressure reflects the presence of similar abnormalities in sympathetic output to other regions of the body. Activation of baroreceptors appear to produce selective changes in efferent sympathetic activity to various vascular regions which are both qualitatively and quantitatively different. Thus, the present studies imply only that there may be an alteration in the neurogenic control of renal function in the sodium depleted state. On the other hand, recent studies from this laboratory and another laboratory suggest that in the dog sodium depletion is accompanied by a generalized decrease in peripheral sympathetic nerve activity.

In summary, restricted dietary sodium intake with diuretic therapy causes in anesthetized dogs a significant decrease in the discharge of renal sympathetic nerves to forced variations in MAP that is reversed by section of the cervical vagus sympathetic trunks. These data lend further support to the hypothesis of a complex disregulation of central and peripheral cardiovascular control following prolonged sodium restriction.

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