Effect of Chronic Sodium Depletion on Cerebrospinal Fluid and Plasma Catecholamines

K. Bridget Brosnihan, Ph.D., Julianna E. Szilagyi, Ph.D., and Carlos M. Ferrario, M.D.

SUMMARY To test the role of central neurogenic factors in sodium-depleted states, cerebrospinal fluid (CSF) norepinephrine, epinephrine, and dopamine were measured in mongrel dogs first on a normal sodium intake (65 mEq sodium/day) and then on a 21-day regime of low sodium diet (4 mEq/day combined with diuretics). Plasma catecholamines were measured in the same group of dogs. Three weeks of sodium depletion supplemented with diuretics caused a 24-fold increase in plasma renin activity, hemoconcentration, and elevated serum protein concentration. Both plasma and CSF sodium decreased significantly. After sodium depletion, plasma norepinephrine rose 76% but epinephrine and dopamine did not change. The same pattern was observed whether samples were obtained in conscious or anesthetized animals. In CSF, norepinephrine rose 44% during sodium depletion, while epinephrine and dopamine remained unchanged. The CSF norepinephrine was related inversely to the CSF sodium concentration and directly to plasma renin activity. These observations support the view that the combined procedure of restricted dietary sodium intake and diuretic therapy causes alterations in CSF norepinephrine in a direction compatible with possible overactivity of central noradrenergic neurons. (Hypertension 3: 233-239, 1981)

KEY WORDS • cerebrospinal fluid • catecholamines • norepinephrine • epinephrine • dopamine, electrolytes • plasma renin activity • sodium depletion

It has been firmly established that maintenance of normal arterial blood pressure in the sodium-depleted state is due to overactivity of the renin angiotensin system.1,2 On the other hand, the effect of sodium depletion on sympathetic nervous system function remains controversial. Plasma norepinephrine (NE) is elevated during sodium depletion,3,4 but this may not reflect an increase in neurogenic activity because other studies using more direct markers of neuronal activity do not support this conclusion. For example, Ljundqvist,7 using a histochemical technique, showed that adrenergic nerve terminals in the rat were depleted of norepinephrine following sodium depletion. Rocchini et al.8 and Brosnihan et al.9 have demonstrated the presence of blunted pressor responses to carotid occlusion in sodium-depleted dogs. In view of these divergent findings, characterization of the contribution of the central nervous system (CNS) to changes in the state of sodium balance seems warranted.

The present study was undertaken to obtain direct evidence in the dog of the effect of chronic sodium depletion on plasma and cerebrospinal fluid (CSF) catecholamines, plasma renin activity (PRA), and vascular reactivity to both norepinephrine and angiotensin II. The neurochemical analysis of CSF is recognized as a method for studying alterations in the CNS metabolism of living subjects, providing valid information about the central rather than the peripheral metabolism of neurotransmitters and hormones.10-12 A comprehensive evaluation of these factors has not been performed in the dog before, in spite of the fact that this animal is used to study hypertensive mechanisms frequently. An additional advantage of the dog versus other species studied is that its larger size permits adequate amounts of CSF and blood to be collected serially for assessment without causing pronounced changes in cardiovascular function due to removal of the fluid.

Methods

Experiments were performed in 26 male mongrel dogs (weight, 19 ± 0.4 kg) placed on a controlled dietary sodium (Na+) intake of either 65 mEq Na+/day (Purina Dog Chow, Petzer Brothers, Bedford, Ohio) or 4 mEq Na+/day (Prescription h/d, W.A. Butler, Warren, Ohio) for 21 days. The degree of sodium depletion was enhanced by supplemental treatment with furosemide (40 mg, i.m., Lasix, Hoechst-Roussel Pharmaceuticals, Somerville, New
Jersey) given for 4 days before the last day of the diet; food and water were held during the 24 hours preceding the experiment.

Blood samples for catecholamines, renin activity, hematocrit, serum protein, and electrolyte concentrations were collected by direct venopuncture in the conscious resting dog. Thirty minutes after induction of pentobarbital (30 mg/kg) anesthesia, a 5 ml sample of CSF was taken by direct puncture of the cisterna magnum with a 20 gauge needle. In 14 of the 26 dogs, an additional venous sample of blood was taken simultaneously with the CSF one. All samples of CSF and blood were immediately frozen at -70°C until assayed.

Pressor responsiveness to vasoconstrictor doses of either angiotensin II ([1-aspartic acid, 5-isoleucine] AII, prepared by Dr. M. Khosla of Cleveland Clinic, dose range: 0.05 to 5 μg) or norepinephrine (Levophed, Winthrop Lab, New York, dose range: 1 to 40 μg) were determined in 12 of the 26 dogs after insertion of catheters into a femoral artery and vein with a sterile procedure. Doses were randomized and spaced 15 minutes apart.

Chemical Methods

Blood (5 ml) and CSF (5 ml) were collected in tubes containing 20 μl/ml of ethylene glycol-bis (β-amino-ethyl ether), N,N'-tetraacetic acid (90 mg/ml), and glutathione (60 mg/ml), centrifuged at 4°C and frozen until assayed at -70°C. Since samples were not treated with perchloric acid, sulfatase, nor lyophilized and then treated with acid to hydrolyze the conjugated catecholamines (as recommended by Buu and Kuchel),

only free catecholamines were measured as described by Johnson et al. Plasma and CSF norepinephrine, epinephrine, and dopamine were measured using the radioenzymatic method described by Peuler and Johnson.

These catecholamine measurements were performed by Upjohn Diagnostics, Kalamazoo, Michigan.) The decarboxylase inhibitor, benzoyloxyamine (0.1 mM), was routinely included in the incubation media to prevent the action of any aromatic amino acid decarboxylase possibly present as a contaminant of the catechol-o-methyl transferase (COMT) preparation; thus, the levels of dopamine reported do not reflect conversion during incubation. Potential substrates for COMT that might migrate with the dopamine metabolite, 3-methoxytyramine (for example, epinephrine and salsolinol), were not rigorously eliminated. However, since epinephrine is likely to exist in trace quantities, it probably does not contribute substantially to the radioactivity migrating with 3-methoxytyramine, and salsolinol has been recently shown to migrate at higher Rf values than 3-methoxytyramine.

The PRA was measured by radioimmunoassay and expressed as nanograms per milliliter per hour of angiotensin I generated according to the method of Haber et al. Total proteins were measured in serum using a Technicon analyzer. Serum and cerebrospinal Na+ and K+ concentrations were measured on a flame photometer (Instrumentation Laboratory, Lexington, Massachusetts). The CSF electrolyte determinations were done in a subgroup (n = 10) of the normal and sodium-depleted dogs.

Statistics

Data were analyzed by Student's t test for paired and nonpaired data. Linear regression analysis and correlation coefficient were calculated by the method of least squares. Differences and correlation coefficients were considered statistically significant when p < 0.05.

Results

Table 1 summarizes the average changes in body weight, plasma, and CSF electrolytes, total proteins, hematocrit, and PRA before and after 21 days of sodium depletion. The main characteristics of sodium depletion were a 24-fold increase in PRA, hyperconcentration, hyponatremia, and elevated serum protein levels.

Figure 1 compares the average concentrations of catecholamines in the plasma of anesthetized versus conscious dogs both before and after sodium depletion. While plasma norepinephrine levels were comparatively higher in samples of peripheral blood from conscious dogs, anesthesia did not abolish the effect of sodium depletion on baseline levels of catecholamines. In normal anesthetized dogs, plasma norepinephrine averaged 127 ± 19 pg/ml and rose to 232 ± 28 pg/ml (p < 0.01) following 21 days of sodium depletion. There was a tendency for plasma epinephrine to rise after sodium depletion but these differences did not attain statistical significance whether measurements were obtained before or after anesthesia. Moreover, the concentration of dopamine in plasma remained unaffected.

<table>
<thead>
<tr>
<th>Table 1 Basal Values of Dogs Placed on a Normal and Low Sodium Diet</th>
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<tbody>
<tr>
<td><strong>Basal value</strong></td>
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<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
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<tr>
<td>Plasma Na+ (mEq/liter)</td>
</tr>
<tr>
<td>Plasma K+ (mEq/liter)</td>
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<tr>
<td>CSF Na+ (mEq/liter)*</td>
</tr>
<tr>
<td>CSF K+ (mEq/liter)*</td>
</tr>
<tr>
<td>Hematocrit</td>
</tr>
<tr>
<td>Total proteins (g/100 ml)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
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</tbody>
</table>

*p < 0.05. Values are x ± 88.

*Subgroup of dogs (n = 10); in all other studies, n = 26.
Sodium depletion of 21-day duration caused a significant rise in CSF norepinephrine but had no effect on either epinephrine or dopamine concentrations (fig. 2). On an average, CSF norepinephrine rose from 87 ± 9 to 126 ± 14 pg/ml (p < 0.05). Moreover, there was no correlation between the increases in plasma and CSF norepinephrine (r = 0.27, p > 0.05). The latter observation is in agreement with previous findings regarding a source other than plasma for the norepinephrine in the CSF.10, 11 In the CSF of normal and sodium-depleted dogs, the concentrations of epinephrine bore no direct proportionality to those in plasma (figs. 1 and 2), whereas the levels of dopamine in CSF and plasma were weakly correlated (r = + 0.37, p < 0.05).

Relationship Between Plasma and CSF Sodium, Catecholamines, and Plasma Renin Activity

The concentration of CSF sodium in the cisterna magna of both normal and sodium-depleted dogs was consistently higher than in the venous plasma. The converse was true for the concentration of potassium (K⁺) ions (table 1). Twenty-one days of sodium depletion were associated with a mild but significant fall in both plasma and CSF Na⁺ concentration without concurrent alterations in K⁺ (table 1).

Moreover, changes in CSF Na⁺ were linearly correlated with plasma Na⁺ (table 2 and fig. 3). To obtain additional information regarding a possible interplay between Na⁺ and the other measured variables, linear regression analyses were performed on the pooled data from normal and sodium depleted dogs. The results are shown in table 2 and figures 4 and 5. In the main, CSF sodium correlated inversely with both CSF norepinephrine (fig. 4) and PRA, but there was no correlation between CSF Na⁺ and plasma norepinephrine (table 2). The CSF norepinephrine correlated with both plasma sodium and PRA. The correlation between CSF norepinephrine and CSF sodium was statistically significant whether the CSF norepinephrine was expressed as either the absolute (r = −0.53, p < 0.025) or the logarithmic (r = −0.51, p < 0.025) value (fig. 4). The PRA correlated significantly with
### Table 2. Correlation between Baseline Values of Normal and Sodium Depleted Dogs

<table>
<thead>
<tr>
<th></th>
<th>CSF Na⁺</th>
<th>CSF NE</th>
<th>Plasma Na⁺</th>
<th>Plasma NE</th>
<th>PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSF Na⁺</strong></td>
<td>0.53</td>
<td>0.62</td>
<td>-0.31</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.025</td>
<td>0.01</td>
<td>(ns)</td>
<td>(0.05)</td>
<td></td>
</tr>
<tr>
<td><strong>CSF NE</strong></td>
<td>-0.31</td>
<td>0.26</td>
<td>(ns)</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.025</td>
<td>(ns)</td>
<td>(0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Na⁺</strong></td>
<td>-1.0</td>
<td>(ns)</td>
<td>(ns)</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>(ns)</td>
<td></td>
<td>(ns)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma NE</strong></td>
<td>0.26</td>
<td>0.51</td>
<td>-0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>(ns)</td>
<td></td>
<td>(ns)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PRA</strong></td>
<td>0.10</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>(ns)</td>
<td></td>
<td>(ns)</td>
<td></td>
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</tr>
</tbody>
</table>

PRA = plasma renin activity, Na⁺ = sodium; NE = norepinephrine.

Correlations where CSF Na⁺ is included are from a subset of 10 dogs; thus *n* = 20 for determinations from both normal and sodium-depleted dogs. For other correlations, 26 dogs were used; thus, *n* = 52. Significance is indicated by the *p* value under the correlation or by (ns) (not significant).

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**Peripheral Vascular Reactivity**

In sodium-depleted dogs, the response curves to the intravenous administration of either angiotensin II (fig. 6 left) or norepinephrine (fig. 6 right) were significantly shifted below those found in normal animals. Comparison between the responses found in the normal and sodium-depleted groups showed that, to obtain a 30 mm Hg change in blood pressure, a 4-times higher dose of angiotensin, but only a 2-times higher dose of norepinephrine, was needed in the sodium depleted dogs.

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**Discussion**

Sodium depletion produced by combined dietary salt restriction and therapy with furosemide produces significant alterations in the concentration of neurohormones participating in blood pressure regulation. This is attested by the increases in PRA and plasma and CSF norepinephrine that correlated with changes in sodium concentration in the CSF compartment. While sodium depletion is known to enhance the activity of the renin-angiotensin system,1,2 the alterations in the CSF content of catecholamines and its relation to CSF sodium have not been reported previously. Following sodium depletion, the content of norepinephrine in the CSF at the level of the cisternal magna rose; the change was correlated with concurrent alterations in CSF sodium and PRA.

Several lines of evidence indicate that most of the norepinephrine in the CSF is central in origin, probably reflecting the activity of noradrenergic cell bodies in the brain stem. First, a direct contribution from plasma is excluded because norepinephrine injected intravenously does not cross the blood-brain barrier.10 This may account for the lack of direct correlation between plasma and CSF content of norepinephrine which we found. Second, measurements of norepinephrine content in various brain regions revealed that the highest concentrations of this neurotransmitter are found in close proximity to the ventricular system.21 Thus, the presence of catecholamines in the CSF is considered to represent release from adjacent brain regions21 with a predominant contribution from the cell bodies in the caudal brain stem.22 On the other hand, Sato and Suzuki23 have shown that adrenergic nerve terminals of cerebral blood vessels make a minor contribution to CSF levels of norepinephrine. This factor may have contributed to the findings of Ziegler et al.16 and Eide et al.17 in man. Although they found that plasma norepinephrine correlated directly with the concentration of the neurotransmitter in the CSF of normal subjects and patients with various disorders, they both subscribe to the view that the correlation may reflect the participation of factors other than plasma contamination.
Additional, albeit indirect, proof for the presence of a blood-brain barrier to catecholamines can be surmised by the differences in the concentration of norepinephrine and epinephrine between the plasma and CSF in the dog. The level of CSF norepinephrine was about 69% of that in plasma, while in the CSF epinephrine was found only in trace amounts. Therefore, we believe that the increased norepinephrine concentration in the CSF of sodium-depleted dogs may reflect increased activity of brain stem noradrenergic neurons, a view that is compatible with previous observations of decreased sympathetic vasomotor activity in the sodium-restricted state.17,18 Van Ameringen et al.14 and Nakamura et al.25 showed that in DOCA-salt hypertensive rats a decrease in brain stem noradrenergic activity was associated with elevation of turnover rates for norepinephrine in heart and kidneys. In our experiments in sodium-depleted dogs, the increase in CSF norepinephrine suggests augmented activity of noradrenergic neurons, but this conclusion must await confirmation with experiments assessing turnover.
rates and/or metabolites. Nevertheless, results of these studies assessing central noradrenergic activity at extremes of sodium metabolism appear to be congruent: 1) decreased turnover rates of brain stem norepinephrine in sodium-loaded animals; and 2) increased CSF norepinephrine in sodium-depleted animals.

The increase in plasma norepinephrine, as shown in the present experiments, has also been observed in humans. Since our findings were obtained whether samples were from conscious or anesthetized animals, it appears that one characteristic of the sodium-restricted state is an increase in plasma norepinephrine even after taking into account the limitation of drawing samples in awake animals or the deleterious effects of anesthesia. Additionally, an elevated plasma norepinephrine has been found whether sodium depletion is achieved by diet, diuretics, or a combination of both. Thus, it is the sodium deficit rather than a nonspecific effect of the diuretic that apparently affects the plasma levels of norepinephrine.

The mechanism responsible for the increase in plasma norepinephrine is unclear. It may reflect a change in pool size. This is supported by the presence of homoconcentration and elevated total plasma proteins, which in another study from our laboratory amounted to an 18% decrease in plasma volume. However, the mild hypovolemia associated with sodium depletion cannot alone be the cause of the increases in plasma norepinephrine, which averaged 46% and 76% above control values in the conscious and anesthetized dog respectively.

Another explanation is that it reflects an increase in neurotransmitter release from sympathetic nerve endings. Against this idea are the studies demonstrating the reduced reflex responsiveness due to carotid occlusion in conscious and anesthetized sodium-depleted dogs. Histochemical studies by Ljundqvist in the rat showed that adrenergic nerve terminals were depleted after sodium depletion. Sybertz and Peach observed a reduced angiotensin-induced potentiation to sympathetic nerve stimulation in the portal vein of sodium-depleted rabbits.

Finally, the rise in plasma norepinephrine may reflect adrenal medulla overactivity, since angiotensin is a potent stimulus for release of adrenal catecholamines. Feuerstein et al showed that after hemorrhage the enhanced adrenal medullary secretion is blocked by either nephrectomy or infusion of an angiotensin antagonist. Moreover, they were able to show a preferential release of adrenal norepinephrine during this angiotensin-mediated stimulation. Hemorrhage, like sodium depletion, is associated with elevated PRA; this may change the profile of circulating catecholamines markedly without any obvious relationship to the level of neurogenic activity. The weak correlation between PRA and plasma norepinephrine found in the experiments may be an indicator of this interplay.

Sodium depletion did not alter the levels of dopamine in plasma and CSF. The major source of plasma dopamine has been shown to be the adrenal medulla. In plasma, dopamine is found predominantly in the conjugated form. In dogs, Kuchel et al. have demonstrated that 99% of dopamine exists as a sulphoconjugate. In our study the levels of free dopamine found in venous plasma is close to that reported by Kuchel et al. in dogs and by Johnson et al. in humans. The lack of change in dopamine during sodium depletion may reflect efficient conjugation for dopamine in dogs. Dopamine sulfate has been found by Tyce et al. in CSF of dogs but free dopamine was not detected, probably because the sensitivity of their assay was too low. Using the radioenzymatic method, we found that the levels of free dopamine in CSF of dogs were similar to those found in plasma.

Decreased vascular reactivity to both angiotensin II and norepinephrine was found in sodium-depleted dogs. Similar findings have been well documented for angiotensin II in other species. Prior occupancy of receptor sites was suggested as a likely possible mechanism, although other changes in receptor affinity may also play a part. There are no definitive studies regarding the effect of sodium depletion on vascular reactivity to norepinephrine. Vasoconstrictor responses to the injection of norepinephrine in sodium-depleted states have been reported unchanged, augmented, or even depressed. The lack of consistent findings in regard to norepinephrine effects on vascular reactivity may in part be related to use of anesthesia, the degree of volume depletion, or the regulatory activity of baroreceptor reflexes.

In summary, this study was prompted by a number of clinical and experimental observations that appeared to contradict the proposed view that sodium depletion predominantly affects the renin-angiotensin system, sparing or even augmenting sympathetic activity and/or reflexes. We conclude that in the dog the combined procedure of restricted dietary sodium intake and diuretic therapy causes alterations in CSF norepinephrine in a direction compatible with possible overactivity of central noradrenergic neurons. The correlations between CSF Na+ and CSF norepinephrine as well as PRA lend support to the hypothesis proposed by Ferrario and McCubbin of an interplay between the renin-angiotensin system, sodium, and the sympathetic nervous system in the regulation of arterial pressure in normal and disease stages. The study raises important questions, the resolution of which awaits further investigation.

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CSF AND PLASMA CATECHOLAMINES DURING SODIUM DEPLETION/Brosnihan et al


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