Catecholamines and Serotonin in the Area Postrema of Normal and Sodium-Depleted Dogs

K. BRIDGET BROSNIHAN, PH.D., CARLOS M. FERRARIO, M.D., JUAN M. SAAVEDRA, M.D., AND ROBERT C. SPETH, PH.D.

SUMMARY Sodium depletion, a maneuver that is accompanied by a 14-fold elevation of plasma renin activity (PRA), alters the norepinephrine concentration of the canine area postrema (AP), a circumventricular organ of the 4th ventricle known to be sensitive to circulating angiotensin II. The norepinephrine concentration of the AP after 3 weeks of sodium depletion decreased by 43%, whereas the concentration of epinephrine and dopamine and the activity of phenylethanolamine-N-methyltransferase (PNMT) did not change. In the pyramidal tract (PT) and choroid plexus (CP) catecholamines were present in significantly lower amounts than in the AP; their concentrations were unaffected by sodium depletion in the PT, but in the CP the norepinephrine concentration was reduced. Serotonin was present in the AP but its concentration was unaltered by sodium depletion. These findings provide evidence that sodium depletion produced an alteration in the concentration of norepinephrine of the area postrema without any change in the concentration of epinephrine, dopamine or serotonin. (Hypertension 3 (suppl II): II-151-II-154, 1981)

KEY WORDS • low sodium diet • norepinephrine • diuretics • epinephrine • dopamine • plasma renin activity

O f the circumventricular organs found in the dog, the area postrema has been shown to play an important role in cardiovascular function. Ferrario et al. and Joy and Lowe discovered that the AP was the region of the medulla which mediates the central action of blood borne angiotensin II. More recently, the AP has been shown to be tonically active in the control of arterial pressure and hemodynamic variability of trained unanesthetized dogs. Although neuroanatomical studies have shown the presence of neurons in the AP, their neurochemical substrate in the dog is not known. The aim of this study was to assess the monoaminergic concentration of the canine AP and to determine whether it is affected by sodium depletion.

Methods

Experiments were performed in 23 male mongrel dogs fed a diet containing either less than 4 mEq Na+/day (9 of 23 dogs) or 65 mEq Na+/day for 21 days as described elsewhere. Animals placed on a low sodium regime were given a diuretic (furosemide, Lasix, Hoechst-Roussel Pharmaceuticals, Somerville, New Jersey) at a dose of 80 mg IM for the last 4 days on the diet. At the completion of either the normal or salt restricted protocols blood (15 ml) was obtained in the conscious dog by venapuncture for the purpose of determining the hematocrit, blood urea nitrogen, serum creatinine and electrolytes. An additional aliquot of blood (5 cc) was taken to assay plasma renin activity by radioimmunoassay.

After anesthesia with sodium pentobarbital (30 mg/kg) the chest was opened and a large bore catheter was inserted into the left ventricle via the apex. The thoracic aorta was cross-clamped just below the subclavian artery and the right atrium was slit open. The animals were given an additional lethal dose of pentobarbital followed by rapid perfusion of the dog's head with chilled, isotonic saline as described previously. At the completion of the perfusion period the lower
brainstem was removed and frozen immediately. Blocks of tissues containing the area postrema, the pyramidal tract (as a control tissue), and choroid plexus were dissected with the aid of an operating microscope. All tissues were stored at −70°C until assayed and at this temperature have been shown to be stable for up to 6 months. The remaining portion of the brainstem tissue was examined histologically to verify the completeness of the dissection. Only tissues in which greater than 50% of the area postrema was removed were included in the study.

Tissue catecholamines (norepinephrine, epinephrine and dopamine) and serotonin were measured by radioenzymatic assays as described by Saavedra et al.9,10 Values were expressed as ng of catecholamines/mg protein of analyzed tissue. Sensitivities of assay are 2 pg for norepinephrine and epinephrine, 5 pg for dopamine and 25 pg for serotonin. Protein level was determined by the method of Lowry et al.11 The enzyme PNMT was measured by the enzymatic isotope method.12 Data are expressed as mean ± standard error of mean. Student’s t test for nonpaired data was used to evaluate statistical significance.

**Results**

Twenty-one days of sodium depletion elicited a 14-fold increase in plasma renin activity accompanied by mild hyponatremia and hemococoncentration (table 1). The area postrema of normal dogs contains higher concentrations of norepinephrine, epinephrine and dopamine than either the pyramidal tract or the choroid plexus (fig. 1). In a subgroup of four normal dogs high concentrations of serotonin (18.6 ± 2.5 ng/mg protein) were found in the area postrema; whereas in the choroid plexus and pyramidal tract serotonin concentration averaged 7.3 ± 0.9 and 6.7 ± 1.4 ng/mg protein respectively.

Sodium depletion for a 21-day period significantly decreased the concentration of norepinephrine in the area postrema by an average of 43% (fig. 1). A similar reduction in the concentration of norepinephrine (58%) was observed in the choroid plexus but tissue levels of norepinephrine in the pyramidal tract although slightly reduced did not change significantly. On the other hand, sodium depletion produced no significant changes in the tissue concentration of epinephrine and dopamine in either the area postrema, the choroid plexus, or pyramidal tract (fig. 1). PNMT, the enzyme responsible for the synthesis of epinephrine, averaged 53.9 ± 7.9 in normal dogs compared to 41.4 ± 8.1 picomoles/mg protein/hr in sodium depleted animals. This difference was not statistically significant. In a subgroup of five sodium depleted dogs the serotonin concentration of the area postrema was 21.8 ± 3.3 ng/mg protein, which did not differ from the value in normal dogs (18.6 ± 2.5 ng/mg protein). Sodium depletion did not alter the serotonin concentration of the CP (6.7 ± 3.1 ng/mg protein) and PT (6.9 ± 2.2 ng/mg protein).

### Table 1. Basal Values of Dogs Placed on Either a Normal or Sodium Depleted Diet

<table>
<thead>
<tr>
<th></th>
<th>Normal Diet</th>
<th>Sodium Depleted Diet (&lt; 4 mEqNa+/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity</td>
<td>1.3 ± 0.5</td>
<td>17.9 ± 4.0†</td>
</tr>
<tr>
<td>Serum Na+ (mEq)</td>
<td>146.0 ± 0.4</td>
<td>143.0 ± 0.8*</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>45 ± 1</td>
<td>53 ± 1*</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg %)</td>
<td>13.0 ± 0.6</td>
<td>15.9 ± 1.1*</td>
</tr>
<tr>
<td>Serum creatinine (mg/100 ml)</td>
<td>0.81 ± 0.05</td>
<td>1.01 ± 0.04*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>22.2 ± 1.0</td>
<td>19.8 ± 0.8</td>
</tr>
<tr>
<td>∆Body weight (kg)</td>
<td>-2.2 ± 0.3</td>
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Results are means ± SEM of data obtained from normal (n = 14) and sodium depleted (n = 9) dogs. The change in body weight is calculated from the weight of the same group of dogs before and after the sodium-depleted diet. *p < 0.05; †p < 0.01.
Discussion

These data indicate that norepinephrine and serotonin are the main monoamines within the dog’s area postrema, a circumventricular organ involved in the central cardiovascular actions of angiotensin II.1,2 Dopamine and epinephrine were also present in the dog’s area postrema but the concentrations of these catecholamines were significantly less than norepinephrine. The presence of PNMT activity in the AP suggests that epinephrine can be synthesized locally. In other species not only PNMT but other synthetic and degradative enzymes, i.e. tyrosine hydroxylase,3 dopamine-beta-hydroxylase,4,5 catechol-0-methyltransferase,6 and monoamine oxidase4,8 activities, have been demonstrated in the AP. These findings imply that catecholamines are not present merely as blood-borne contaminants but as integral parts of the neuronal structure of the area postrema. The biochemical profile of monoamines found in the dog’s area postrema has not been reported previously but it appears to be similar to that reported for the area postrema of the rat,6,9 although a functional role for the area postrema in the rat has yet to be established.

Sodium depletion was associated with a significant reduction in the norepinephrine concentration of the area postrema with no alterations in dopamine, epinephrine, serotonin, or PNMT activity. Because sodium depletion did not alter norepinephrine concentration in the pyramidal tract, these data suggest that the change in norepinephrine concentration in the area postrema is specific in nature and may represent an alteration in the noradrenergic activity of or onto this structure. It is possible that these changes are due to the increased activity of the renin angiotensin system.

It is unlikely that the alterations in tissue norepinephrine found in the area postrema represent blood contamination. This possibility had to be taken into account since circumventricular organs like the area postrema are highly vascular structures and also possess a deficient blood brain barrier. First, the perfusion technique employed in these experiments was effective in removing all noticeable traces of blood within the circulation of the dog brain.10 In addition we have shown previously that blood levels of norepinephrine of sodium-depleted dogs are less than 0.5 ng/ml,4 which on a volume basis is less than 2% of the tissue norepinephrine concentration (assuming 100 mg protein/ml brain volume). These findings indicate that plasma levels could not be responsible for the changes observed in the tissue concentration of norepinephrine and that a local factor accounted for the alterations in the tissue content of this biogenic amine. The presence of norepinephrine in the area postrema may reflect its origin from cell bodies within the confines of the area postrema or axons and terminals with cell bodies immediately adjacent to the area postrema. Both possibilities are likely. In the rat Torack et al.11 used immunofluorescent techniques to demonstrate that A2 cell bodies contributed heavily to noradrenergic innervation of the area postrema. However, they also showed numerous norepinephrine-containing cell bodies within the area postrema itself. The high concentration of serotonin in the canine area postrema confirms the earlier finding by Saavedra12 in the rat and contrasts with the findings of Fuxe and Owman13 who were not able to detect serotonin in the dog’s area postrema using a histofluorescence technique.

The decreased norepinephrine concentration of the AP cannot be equated with the change in noradrenergic activity of postganglionic neurons innervating the vasculature of the area postrema nor with the change in noradrenergic activity of the choroid plexus. To begin with, Fuxe and Owman13 showed that bilateral cervical sympathectomy did not result in any discernible change in the monoamine-specific fluorescence found in nerve cells or terminals within the area postrema of rabbits and cats. Second, capillaries are the predominant microvascular components of the area postrema14 and as such would not possess a substantial sympathetic innervation. Finally, because the innervation of the choroid plexus originates in the superior cervical ganglion,15 the decrease in norepinephrine concentration of the choroid plexus found in our experiments may reflect an alteration in postganglionic sympathetic activity. Although the exact interpretation of this finding is not readily explainable, it is consistent with the observation by Ljungqvist16 of partial or complete disappearance of transmitter from cardiac and renal nerve terminals in sodium depleted rats. The decreased content of norepinephrine in a vascular bed innervated by postganglionic sympathetic fibers provides further evidence for the possibility that sodium depletion is associated with significant alterations in the activity of both the central and peripheral sympathetic control of the circulation.17,18

The decrease in area postrema norepinephrine as a result of sodium depletion is intriguing and congruent with the possibility that alterations in sodium metabolism may affect central nervous system noradrenergic activity. Previous studies by Ferraro and colleagues19,20 have pointed out the need to re-evaluate traditional concepts regarding the effect of sodium depletion on cardiovascular function and the autonomic nervous system. In the dog sodium depletion causes blunting of sympathetic reflexes due to an abnormal interaction between the carotid sinus and the low pressure baroreceptor system.21 Moreover, the concentration of norepinephrine in the cerebrospinal fluid at the level of the cisterna magna increased in sodium depleted dogs.6 These results suggest an abnormality in the release and/or uptake of neuronal norepinephrine in regions of the medulla oblongata involved in regulation of cardiovascular function after chronic sodium depletion.6

Alterations in sodium metabolism have been reported to affect brainstem noradrenergic turnover,22 and the suggestion has been made that changes in the activity of CNS noradrenaline and adrenaline pathways might participate in the pathogenesis of experimental hypertension.23 How well plasma, CSF and tissue norepinephrine concentrations reflect neuronal
release of transmitters is open to question; however, additional measurements of turnover rates and/or metabolites in the area postrema and other brainstem nuclei of sodium depleted dogs are needed to determine the nature of the mechanism that accounts for the alteration in the norepinephrine concentration of this brain-stem region.

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