Increased noradrenaline content of hypothalamic nuclei in association with worsening of hypertension after high sodium intake in the young spontaneously hypertensive rat

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Summary

1. High sodium intake results in an exacerbation of hypertension accompanied by evidence of increased peripheral sympathetic activity in the young spontaneously hypertensive rat (SHR) of the Okamoto strain.

2. To examine the hypothesis that high sodium intake increases peripheral sympathetic activity via an influence on central noradrenergic pathways involved in cardiovascular regulation, the effect of dietary sodium intake on noradrenaline stores of individual hypothalamic nuclei was examined in young SHR.

3. After 2 weeks of high sodium intake, the noradrenaline content of the anterior and dorsomedial hypothalamic nuclei of SHR was increased when compared with SHR receiving normal sodium intake. Increases in the noradrenaline content of anterior hypothalamic nucleus persisted at 4 weeks. No changes were seen in other regions examined.

4. These observations lend support to the hypothesis that sodium and the sympathetic nervous system have synergistic effects in the pathogenesis of hypertension in the SHR.

Key words: central noradrenergic activity, hypothalamus, noradrenaline, sodium, sympathetic nervous system.

Introduction

Studies in two sodium-dependent models of experimental hypertension, the DOCA/salt hypertensive rat and Dahl salt-sensitive hypertensive rat, have suggested that there may be important sodium–neural interactions in the pathogenesis of hypertension. In both models hypertension does not develop in the absence of sodium excess, and in both there is evidence of increased sympathetic nervous system activity [1, 2]. Studies in the DOCA/salt model have shown that noradrenaline turnover is increased in peripheral organs and decreased in brain stem [1, 3]. In recent experiments brain-stem noradrenaline turnover has been shown to be independent of blood pressure, but returned to normal when normal sodium balance was restored [3]. These findings suggest that the state of sodium balance per se may influence central noradrenergic activity in the rat.

The spontaneously hypertensive rat (SHR) of the Okamoto strain is considered by most investigators to be a neurogenic model of experimental hypertension [4–10]. The influence of sodium on the development and maintenance of hypertension in this model is less well defined. Severe sodium restriction does not prevent the development of hypertension, but a number of investigators have shown that high sodium intake results in an exacerbation of hypertension in SHR [11, 12]. In addition, there is evidence that increased renal sympathetic activity in SHR may contribute to the pathogenesis of hypertension via an increase in urinary sodium retention [13].

Recent experiments performed in our laboratory and by other investigators have demonstrated increased peripheral sympathetic activity accompanying the sodium-induced increase in blood pressure in SHR [14–17]. In our studies, 11 week old male SHR studied 4 weeks after initiation of high sodium diets had increased plasma noradrenaline levels and an
exaggerated response to ganglionic blockade when compared with SHR receiving normal sodium intake. These findings and those of the previously cited studies suggested the hypothesis that high sodium intake increases peripheral sympathetic activity via an influence on central noradrenergic pathways involved in cardiovascular regulation. The current study was designed to examine this hypothesis.

**Methods**

Animals used in these experiments were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). They were housed in a room with constant temperature (24 ± 1°C) and humidity (60 ± 5%), that was lighted from 07.00 to 19.00 hours. Systolic blood pressures were measured in conscious pre-warmed rats using the tail cuff method.

Male SHR were placed on a diet of either normal (0.29%) or high (3.4%) sodium content beginning at 7 weeks of age. Blood pressure was determined weekly thereafter. Subsets of animals from each dietary group were killed at 2 and 4 weeks after initiation of diets. Animals were killed by decapitation without anaesthesia and the brains removed immediately and frozen in powdered dry ice. Brains were placed in a cryostat at −10°C and 300 μm sections made through the hypothalamus. Individual nuclei were removed with a hollow thin-walled needle, 0.5 mm in diameter, by the method of Palkovits [18]. Punches were stored at −80°C until the time of assay.

For determination of noradrenaline content, 100 μl of perchloric acid (0.4 mol/l) was added to each sample and the tissue disrupted by sonication. A portion (25 μl) was removed for protein determination by a modification of the method of Lowry [19]. Noradrenaline concentration of the remaining homogenate was determined with high performance liquid chromatography with electrochemical detection, a modification of the method of Keller et al. [20] being used. Pooled bilateral punches from a single rat were used for each determination. Results were expressed as ng/mg of protein.

Statistical analysis of the data was performed by using Student's unpaired t-test [21]. Differences were considered significant if $P < 0.05$.

**Results**

Increased sodium intake resulted in a marked increase in the severity of hypertension in young SHR. Blood pressure of animals receiving high sodium intake was significantly greater than that of controls consuming normal sodium intake at both 2 weeks (167 ± 4.9 normal sodium vs 191 ± 4.0 mmHg high sodium, $P < 0.005$) and 4 weeks (175 ± 2.6 normal sodium vs 215 ± 3.0 mmHg high sodium, $P < 0.001$) after initiation of the diets.

Shown in Fig. 1 are the results of measurements of the noradrenaline content of the posterior (PHA), ventromedial (VMH), dorsomedial (DMH), paraventricular (PVH) and anterior (AHA) hypothalamic nuclei of SHR receiving either normal or high intake of sodium beginning at 7 weeks of age. $P$ values represent differences from normal sodium content (unpaired t-test).

**Discussion**

A number of studies have shown that blood pressure in the SHR is sensitive to dietary sodium excess [11, 12, 17]. Recent experiments in our laboratory as well as those of other investigators have focused on the influence of dietary sodium intake on the sympathetic nervous system in this model [14–17]. Dietz and colleagues have shown that in stroke prone SHR receiving high dietary
sodium intake, plasma noradrenaline was increased compared with controls receiving normal dietary sodium, and that these differences became more marked after stress due to cold exposure [15]. In control Wistar–Kyoto (WKY) rats dietary sodium intake did not influence circulating catecholamines. In addition, haemodynamic studies have shown that increased sodium intake results in enhanced total peripheral resistance in SHR but not in control WKY rats [17].

The findings of the current study indicate that sodium intake may influence central as well as peripheral noradrenergic activity. Noradrenaline content of hypothalamic nuclei was used as an index of central noradrenergic activity. Increases in the noradrenaline content of anterior and dorsomedial hypothalamic nuclei were observed in animals receiving high sodium intake. The observation that increases in noradrenaline content occurred only in these two nuclei and not in all regions examined suggests that the changes in noradrenaline content were not a nonspecific effect of sodium on central noradrenergic stores. Both the anterior and dorsomedial hypothalamic nuclei have been implicated in cardiovascular regulation. It has been demonstrated by both radioautographic and electrophysiological means that these nuclei receive projections from the nucleus tractus solitarii, the termination of the primary baroreceptor afferents [22, 23]. In addition, lesions of the anteromedial hypothalamus result in fulminating hypertension characterized by an increase in total peripheral resistance and a fall in cardiac output, providing further evidence that this area, or fibres passing through it, has important cardioregulatory functions [24].

We recognize the limitations of the use of noradrenaline content alone as an index of central noradrenergic activity. However, recent reports have shown that changes in noradrenaline content are paralleled by changes in tyrosine hydroxylase activity [25, 26]. As tyrosine hydroxylase is the rate-limiting enzyme in noradrenaline production, the parallel changes in its activity and noradrenaline content suggest that content may reflect noradrenaline synthesis. Additional studies, including examination of noradrenaline synthesis and turnover, are needed to better define the influence of sodium on central sympathetic activity in this model.

In summary, these studies provide evidence that sodium and the sympathetic nervous system may have synergistic effects in the pathogenesis of experimental hypertension and suggest that the increase in peripheral sympathetic activity seen in SHR after high sodium intake results from an alteration in central sympathetic outflow.

References


