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Enhanced pressor response to centrally administered vasopressin in WKY rats on high sodium diet

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Abstract. Four week old Wistar-Kyoto (WKY) rats were divided into two groups. The experimental group (n=7) was receiving a high sodium diet (3.28% Na) and the control group (n=7) a normal sodium diet (0.22% Na). After 8 weeks, subjects were chronically implanted with the lateral cerebral ventricle (LCV) cannulas and with the femoral artery catheters. Three series of experiments were carried out on the experimental and control groups. In each series mean arterial pressure (MAP) and heart rate (HR) were recorded for 10 min before and 30 min after the LCV infusion. In series 1 artificial cerebrospinal fluid (aCSF) was administered (2 µl/15 s). In series 2 AVP was infused (20 ng/2 µl aCSF/15 s). In series 3 V1a receptor antagonist (V1 ANT), d(CH2)5[Tyr(Me)2,Ala-NH29]AVP, was applied (80 ng/µl aCSF/15 s). There was no difference in baseline MAP and HR between the experimental and control groups. LCV infusion of aCSF had no effect on MAP and HR. LCV infusion of AVP produced a significant increase of MAP, which was greater in the group on the high sodium diet than in the group on normal sodium diet. The experimental group showed a longer hypertensive effect of centrally applied AVP in comparison to the control. LCV administration of V1 ANT did not exert a significant effect on circulatory parameters. These results suggest that the prolonged high sodium diet does not induce hypertension in WKY rats, but it enhances the pressor function of the central vasopressinergic system.

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Key words: high sodium diet, blood pressure, vasopressin, Wistar-Kyoto WKY rats

INTRODUCTION

A high sodium diet is considered to be one of the risk factors in hypertension, however, the mechanisms involved are still not clear. It is known that central regulation of the cardiovascular system plays an important role in the control of arterial blood pressure. Brain vasopressin (AVP) is one of the most potent neurotransmitters regulating haemodynamics. It has been shown that the central AVP enhances hypovolemic bradycardia acting through the brain V1 receptors (Budzikowski et al. 1996), stimulates the sympathetic system (Johnson et al. 1988) and increases the resting blood pressure (Paczwa et al. 1997, Pittman et al. 1982, Stępniakowski et al. 1994). It was also demonstrated that peripheral osmotic stimulation leads to central and peripheral release of AVP (Burnard et al. 1983, Demotes-Mainard et al. 1986, Szczepańska-Sadowska et al. 1983). Other studies have shown that the increase of sodium in cerebrospinal fluid (CSF) results in elevated blood pressure (Huang et al. 2001, Kawano et al. 1991). A high sodium diet is known to change the expression of AVP and V1 receptor mRNA in the rat's brain. Namely, rats on high sodium diet have enhanced AVP and V1b mRNA expression in supraoptic and paraventricular nuclei (Zemo and McCabe 2001) as well as increased expression of AVP mRNA in the hypothalamus (Morita et al. 2001).

The aim of the present study was to determine the influence of a prolonged high sodium diet on the central pressor effect of vasopressin in Wistar-Kyoto (WKY) rats.

METHODS

The experimental series were performed on 14 WKY rats. After the breast-feeding period, 4 weeks old WKY rats were divided into two groups. The experimental group (n=7) was placed on high sodium (3.28% Na), solid diet and the control group (n=7) on normal sodium (0.22% Na), solid diet for over 9 weeks. Both groups had free access to tap water. Twelve weeks old WKY rats were subjected to implantation of a stainless steel cannula into the lateral cerebral ventricle (LCV) (Budzikowski et al. 1996). One week later an arterial catheter was inserted through the femoral artery into the abdominal aorta below the branching of the renal vessels. Both procedures were performed under general anesthesia with chloride hydrate (360 mg/kg i.p.). Three experimental series were performed on freely moving rats 24 hours after the arterial catheterization, during three subsequent days. For all subjects the order of series was assigned randomly. In each of three experimental series mean arterial pressure (MAP) was recorded on-line through the arterial catheter connected to the blood pressure gauge (Biopac MP100 unit). Heart rate (HR) was calculated from systolic peaks. In series 1, MAP and HR were recorded for 10 min. Subsequently the artificial cerebrospinal fluid (aCSF) was administered (2 µl/15 s) into the LCV. During the next 30 min measurements were continued. In series 2 LCV infusion of AVP (20 ng/2 µl aCSF/15 s) was performed 10 min after the onset of the experimental session, and the MAP and HR were recorded for the following 30 min. In series 3, V1a receptor antagonist (V1 ANT), d(CH2)5[Tyr(Me)2,Ala-NH29]AVP

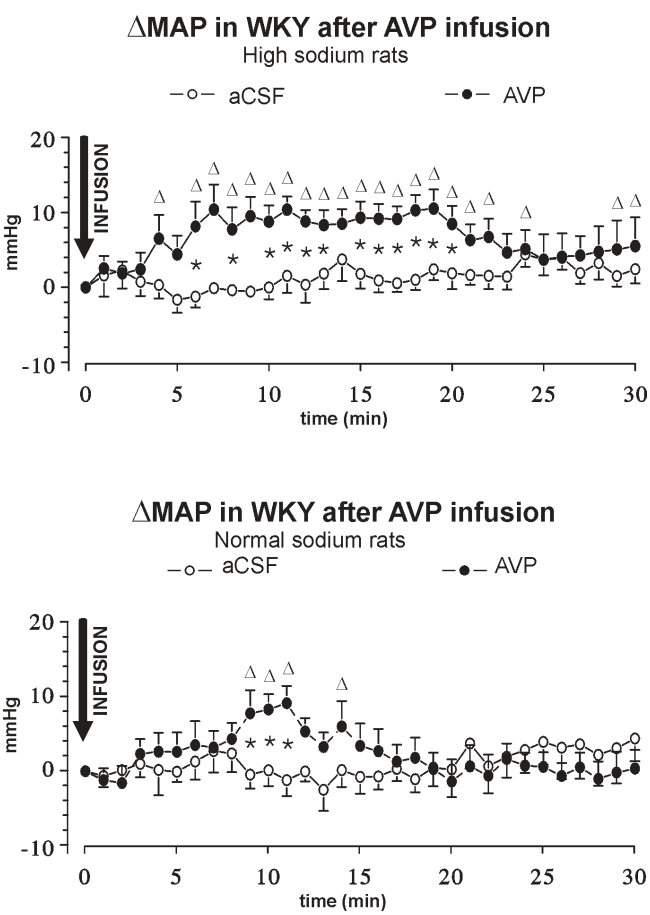
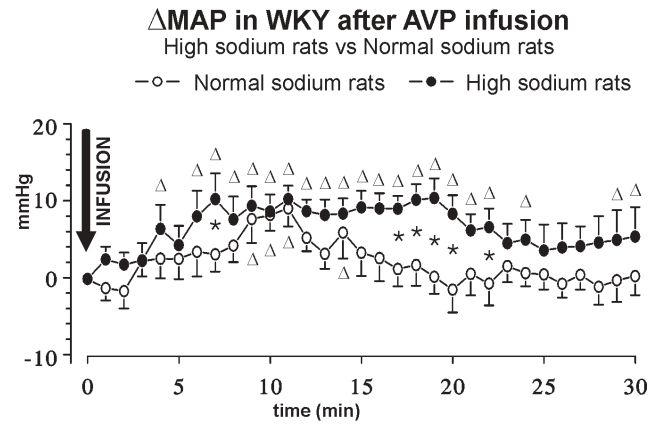
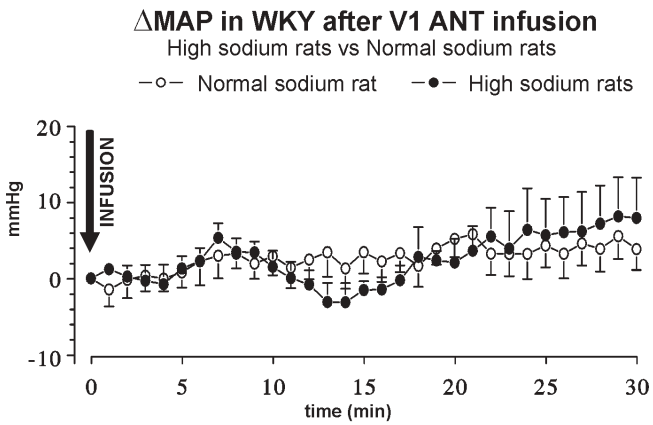
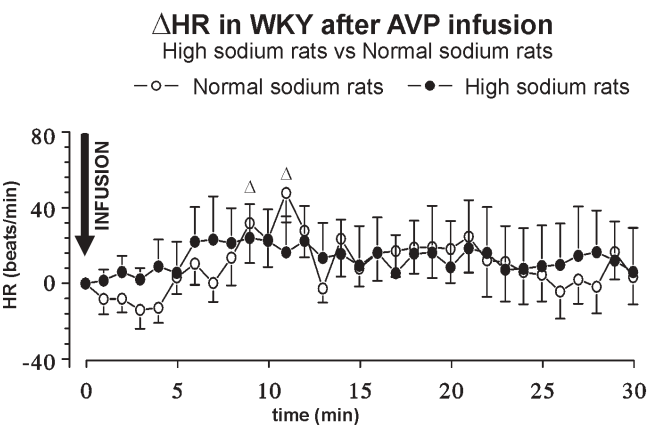


Fig. 1. Changes of mean arterial pressure (ΔMAP) from baseline value after intracerebroventricular infusion of AVP or aCSF in high sodium and normal sodium groups. *P<0.05 control *versus* AVP; Δ P<0.05 *versus* baseline.







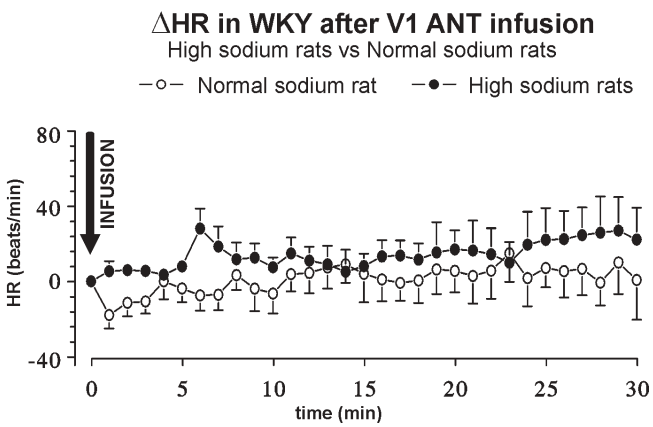


Fig. 2. Changes of mean arterial pressure (ΔMAP) and heart rate (ΔHR) from baseline value in high sodium *versus* normal sodium groups after intracerebroventricular infusion of AVP. **P*<0.05 high sodium rats *versus* normal sodium rats; Δ *P*<0.05 *versus* baseline.

Fig. 3. Changes of mean arterial pressure (ΔMAP) and heart rate (ΔHR) from baseline value in high sodium *versus* normal sodium groups after intracerebroventricular infusion of V1 ANT

(Manning et al. 1992), was applied (80 ng/2 µl aCSF/15 s) after initial 10 min recording and MAP and HR were monitored during the following 30 min. Location of the LCV cannula in the lateral ventricle was confirmed *post mortem* using Evan's blue for staining the ventricular system. Blood for plasma samples was obtained from each animal through the femoral catheter. The blood was centrifuged, plasma samples were frozen and subsequently analyzed for Na and K concentration and osmolality.

The experimental design was approved by the Ethical Committee of the Medical University of Warsaw.

All values are expressed as means with standard error. Analysis of variance (ANOVA) for repeated measurements and Student's *t*-tests were used for appropriate data. In each series, values after LCV injections were compared with pre-injection values by single-factor ANOVA for repeated measurements. The differences between treatments or normal and high sodium groups were evaluated by factorial ANOVA on differences from baseline. The multiple comparisons and t-tests were used to determine significant differences between individual means. Statistical significance was set at $P<0.05$.

RESULTS

Resting MAP and HR were comparable between groups (Table I). Administration of aCSF had no effect on MAP and HR in comparison to the pre-injection values. LCV administration of AVP elicited a signifi-

Table I

Baseline values of MAP and HR in WKY rats before treatment

	Normal Sodium Group		High Sodium Group	
	MAP	HR	MAP	HR
aCSF	115.9 ± 5.3	334.1 ± 19.7	112.7 ± 2.5	318.6 ± 27.9
AVP	118.5 ± 5.8	342.7 ± 20.1	116.1 ± 3.8	340.2 ± 29.3
V1 ANT	120.8 ± 4.1	346.9 ± 24.3	118.3 ± 3.8	346.0 ± 37.7

Means ± SEM

cant increase of MAP in animals on high and normal sodium diet, with $P<0.001$ and $P<0.01$, respectively (one-way ANOVA, Fig. 1). The rise of MAP in the group on the high sodium diet was more pronounced and lasted longer than in the group on the normal sodium diet with significance reaching $P<0.05$ by ANOVA for repeated measurements between 15–25 min (Fig. 2). HR showed a tendency to increase in both groups after LCV infusion of AVP, but did not reach acceptable significance between the group on high sodium diet and the group on normal sodium diet (Fig. 2). LCV infusion of V1 ANT exerted only nonsignificant effects on MAP and HR (Fig. 3), both in animals on high and normal sodium diet. The concentration of sodium and potassium ions in plasma, as well as osmolality were comparable between groups (Table II).

Table II

Plasma osmolality, sodium and potassium plasma concentration

	Normal Sodium Group	High Sodium Group
Osmolality (mOsm/kg)	292.1 ± 3.72	295.0 ± 7.9
Sodium (mmol/l)	139.4 ± 1.2	143.0 ± 1.3
Potassium (mmol/l)	5.0 ± 0.1	4.5 ± 0.4

Means ± SEM

DISCUSSION

In the present study the LCV administration of AVP elicited a significant increase of MAP, which is in accordance with previous findings (Flynn et al. 2002, Pittman et al. 1982, Stępniakowski et al. 1994, Ufnal and Żera 2002). The new finding is that the increase was more pronounced and lasted apparently longer in rats fed the high sodium diet. One of the possible mechanisms responsible for the enhanced response to the centrally administered AVP is an elevated concentration of Na ions in CSF. LCV infusions of NaCl solutions are known to produce rise of MAP (Huang et al. 2001, Kawano et al. 1991). It was also shown that septal and intraventricular release of AVP in the brain is stimulated by systemic administration of hypertonic saline (Burnard et al. 1983). Previous studies have demonstrated that the high sodium diet only transiently increases Na concentration in cerebrospinal fluid in WKY and spontaneously hypertensive rats (Mozaffari et al. 1990), however the coexistence of MAP elevation with the increase in CSF Na concentration was not present in the WKY strain. In the present study, plasma osmolality and plasma K, Na concentrations were comparable between groups after 9 weeks of specific diets. Although plasma analyses revealed slightly higher sodium concentration in the group fed the high sodium chow in comparison with the control group, the difference did not reach acceptable significance. It is known that in healthy subjects plasma sodium concentration may be maintained at the same level in spite of significant increase in sodium intake. This is possible because of simultaneous increase in water intake and water retention. Because CSF sodium concentration reaches an equilibrium with plasma Na in relatively short time (Szczepańska-Sadowska et al. 1983, Szmydynger-Chodobska et al. 1990, Thrasher et al. 1980), it may be assumed that in the present study the CSF Na concentrations did not differ in the rats on high and regular

sodium diet, similarly as the plasma Na levels. Thus, the results of the present study cannot support the assumption that the observed prolonged hypertensive response to AVP resulted from changes of activity of the cardiovascular neurons because of an increased concentration of Na ions in CSF. However, it should be noted that even minute disturbances of Na concentration in CSF may trigger changes in the neuronal activity (Joynt 1966, Mason 1980) and that the schedule of blood withdrawal used in the present study could not detect temporary changes in CSF Na concentration.

In this light it is well recognized that the rats demonstrate the nocturnal feeding rhythm activity. Therefore the highest sodium intake takes place during the dark period of normal day-night cycle. Thus, the fact that in the present and in most of the other studies the blood for plasma sample analysis was obtained during the light period does not exclude the possibility that some significant increase in sodium concentration could occur during the night. In addition the absence of changes in sodium concentration in the extracellular fluid does not exclude changes in intracellular sodium concentration which may play significant role in excitability of the cardiovascular neurons. In the present study it was decided to increase daily ingestion of sodium by using the high sodium solid diet, since this mode of sodium administration was used in many other studies and it appears to be more close to the high sodium intake under natural conditions than the high sodium intake produced by ingestion of large volumes of sodium containing fluid.

Increasing evidence indicates altered expression of vasopressin receptors and brain content of AVP in several models of hypertension. It was shown that spontaneously hypertensive rats have different binding densities of V1a receptors in specific brain regions in comparison to their parental WKY strain (McDougall et al. 2000). It is also known that the high sodium diet increases blood pressure in spontaneously hypertensive rats. It may be hypothesized that the high sodium diet, which enhances hypertensive response of cardiovascular system to vasopressin, may have an effect on expression of vasopressin receptors. Zemo and McCabe (2001) have found that salt-loading results in an increased expression of mRNA for AVP and V1b receptors in supraoptic and paraventricular nuclei as well as in the choroid plexus. Others have found that rats drinking a 2% NaCl solution have a lower content of AVP in the hypothalamus, striatum and cortex, which is accompanied by an increased expression of AVP mRNA in these areas, however no changes in V1a receptor mRNA were found in the examined brain regions (Morita et al. 2001). These findings provide evidence for complex alterations in activity of central vasopressinergic network under conditions of increased sodium intake. Finally, it should be noted that the high sodium intake may increase pressor responsiveness to vasopressin by affecting the other neurotransmitter systems. Several lines of evidence suggest extensive cross-talk between the vasopressinergic and the angiotensinergic neurons, which play significant role in stimulation of release of AVP and at the same time excitation of the cardiovascular neurons (Ferguson and Washburn 1998, Veltmar et al. 1992). Therefore further studies need to be undertaken in order to elucidate mechanisms of enhanced pressor response to AVP in the rats on increased sodium intake.

In the present study, infusion of V1 ANT exerted no effect on resting MAP and HR in animals on normal and high sodium diet. V1 receptors antagonist administered in the present investigation demonstrates selectivity to V1a receptor subtype. Thus, our results indicate that V1a receptors are not involved in regulation of baseline blood pressure in rats on regular and high sodium diet. This finding is in accordance with previous results indicating that central vasopressin in normotensive rats is not involved in cardiovascular regulation under resting conditions, although it does play such a role in certain models of hypertension (Budzikowski et al. 1996, Szczepańska-Sadowska et al. 1998). For instance in renin transgenic rats blockade of central V1a receptors decreases baseline blood pressure (Szczepańska-Sadowska et al. 1998). The finding that in the group on high sodium diet V1a receptors blockade changes MAP and HR only non-significantly may suggest that the high sodium diet does not result in an enhancement of the baseline stimulation of V1a receptors on the cardiovascular neurons, but rather increases their responsiveness to the elevated concentration of vasopressin in CSF.

CONCLUSION

The present study provides evidence that a prolonged high sodium diet sustained over a period of 9 weeks after weaning does not induce hypertension in WKY rats, but it enhances pressor response to centrally administered AVP.

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