0014-4754/95/030227-03$1.50 + 0.20 © Birkhäuser Verlag Basel, 1995

Plasma adenosine concentrations are elevated in Dahl salt-sensitive rats

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Received 6 October 1994; accepted 12 October 1994

Abstract. We measured plasma levels of adenosine in Dahl salt-sensitive rats (DS) and Dahl salt-resistant rats (DR) to examine the potential role of adenosine in cardiovascular regulation in this type of hypertension. Plasma adenosine concentrations were significantly higher in DS than in DR. The NaCl content in the diet did not affect plasma adenosine concentration in either DS or DR. Significant positive correlation was found between adenosine concentrations and systolic blood pressure when the data for DS and DR were analyzed together. These results suggest that adenosine may play an important role in the pathophysiology of hypertension in DS.
Key words. Adenosine; Dahl salt-sensitive rats; high-performance liquid chromatography; hypertension.

Adenosine is an endogenous substance which has a variety of effects on the cardiovascular system. It acts at various sites related to the circulatory system, including the central neurotransmission, autonomic ganglionic transmission, the regulation of glomerular filtration rate and renin release[1-3]. Within the vascular wall, adenosine reduces the release of norepinephrine from adrenergic nerves and relaxes vascular smooth muscle[4]. These actions of adenosine may possibly contribute to the regulation of blood pressure and the pathogenesis of hypertension. We have recently reported that plasma adenosine levels are significantly higher in conscious spontaneously hypertensive rats (SHR) with established hypertension than in age-matched Wistar-Kyoto rats (WKY) and suggested that adenosine may be at least partly involved in the pathophysiology of high blood pressure in SHR[5]. In this study, we measured plasma adenosine concentration of Dahl salt-sensitive rats (DS) and Dahl salt-resistant rats (DR) in order to examine the role of adenosine in this type of hypertension.

Materials and methods

Animals. Four-week-old DS (n = 28) and DR (n = 26) were purchased from Japan Charles River Inc. (Atsugi, Japan). DS and DR were each divided into two groups and were placed on either a high (8%) or a low (0.3%) salt diet. At the age of 10 weeks, systolic blood pressure (SBP) was measured by the tail cuff method. Under pentobarbital anaesthesia (40 mg/kg, i.p.), blood samples (2.0 ml) were withdrawn from the abdominal aorta into a plastic syringe which had been pre-loaded with 1.2 ml ice-cold heparinized saline solution containing 0.01% dipyridamole and 10 mM $MnCl_2$. All procedures were in accordance with the guidelines of the Animal Experiment Committee of the University of Tokyo.

Adenosine assay. We used a specific and sensitive assay for adenosine based on fluorescent determination of adenine compounds by high-performance liquid chromatography (HPLC). Collected blood samples were immediately centrifuged at 3000 rpm for 5 min and 1.5 ml of the supernatant fluids were applied to Sep-Pak C_{18} cartridges which had been washed with 5 ml acetonitrile followed by 10 ml distilled water. After loading, the Sep-Pak C_{18} cartridges were washed with 10 ml distilled water and adenosine was eluted with 3 ml of 20% acetonitrile in water. The eluent was evaporated to dryness and then taken up in 0.25 ml of 0.1 M phosphate buffer (pH 7.0). For preparation of the 1,N^6-etheno derivatives, 50 µl of the solution was heated with 5 µl of 1.9 M bromoacetaldehyde at 85 °C for 20 min. Standard solutions of adenosine (1.5 ml) were treated identically. HPLC was performed using a column (25 cm × 2.1 mm) of Hitachi gel No. 3013 which was made of porous polystyrene polymer beads for adsorption (Hitachi, Tokyo, Japan) and maintained at 30 °C. The mobile phase consisted of 0.1 M phosphate buffer (pH 7.0) and the elute was monitored by a fluorescence spectrophotometer FP-10 (Shimadzu, Kyoto, Japan). In the spectrophotometer, an excitation wavelength of 254 nm was used and the fluroescence at 400 nm was collected by a concave emission grating. Four µl of reaction mixture was injected to the column and fluorescence was monitored for 40 min. Concentrations of adenosine in the sample were determined by comparing peak area ratios to those obtained with authentic adenosine. The data are expressed as mean ± SEM and analyzed by Student's unpaired *t*-test.

Results

Body weight (b.wt) and SBP of 10-week-old DS and DR are shown in the table. SBP of DS on a high salt

Table. Body weight and systolic blood pressure of 10-week-old Dahl salt-resistant (DR) and Dahl salt-sensitive (DS) rats

Group	n	Body weight (g)	Systolic blood pressure (mmHg)
0.3% DR	14	343 ± 5	133 ± 1
8% DR	14	302 ± 6	136 ± 2
0.3% DS	12	348 ± 4	147 ± 3**
8% DS	14	308 ± 1	207 ± 8*,**

Values are given as means ± SEM. *$p < 0.01$ vs 0.3% DS, **$p < 0.01$ vs 0.3% DR and 8% DR.

diet was 207 ± 8 mmHg, and was significantly higher than those of the other three groups of rats ($p < 0.01$). SBP of DS on a low salt diet was 147 ± 3 mmHg and was significantly higher than the SBP of DR on either diet ($p < 0.01$). As shown in figure 1, plasma adenosine concentration in DS on a high salt diet was the highest (0.559 ± 0.091 μM) and significantly higher than the concentrations in DR on either diet (0.03% DR 0.322 ± 0.048 μM, 8% DR 0.244 ± 0.024 μM; $p < 0.05$). Plasma adenosine concentration in DS on a low salt

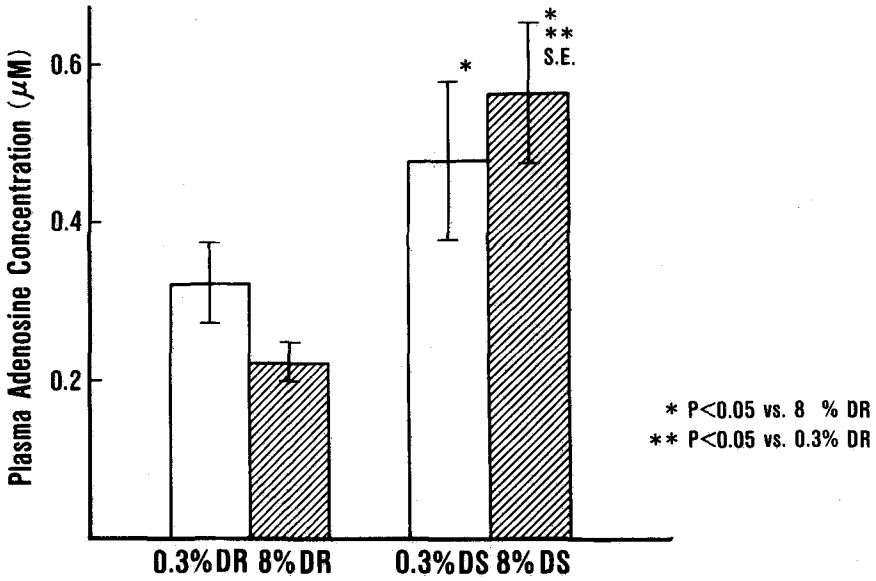

Figure 1. Plasma adenosine concentration in 10-week-old Dahl salt-resistant rats (DR) and Dahl salt-sensitive rats (DS) fed a low (0.3%) salt or a high (8%) salt diet for 6 weeks.

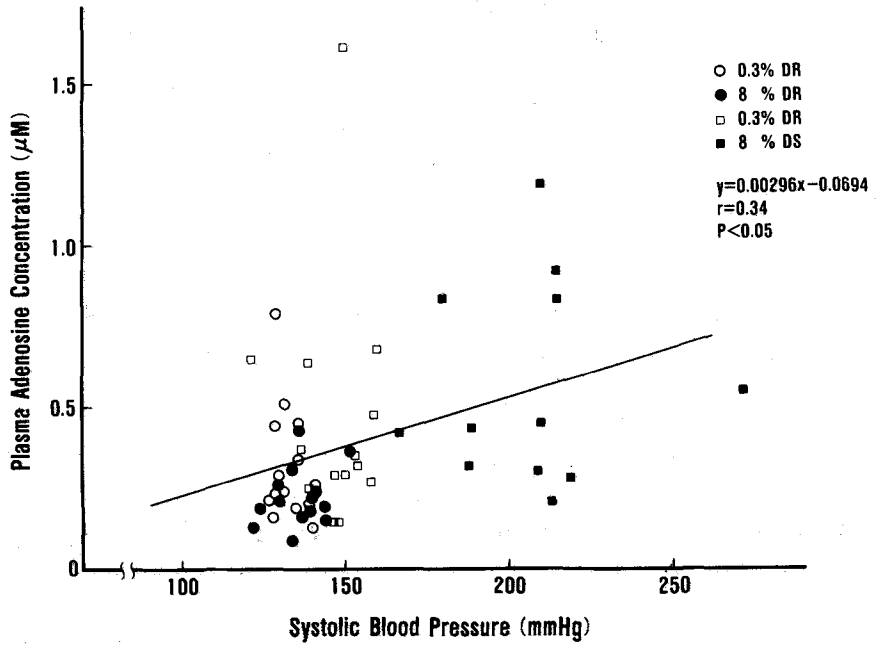

Figure 2. Correlation between plasma adenosine concentrations and systolic blood pressure in Dahl salt-resistant rats (DR) and Dahl salt-sensitive rats (DS).

diet (0.467 ± 0.100 μM) was significantly higher than that in DR on a high salt diet ($p < 0.05$). The salt content in the diet did not affect plasma adenosine concentration in either DS or DR. Significant positive correlation was found between plasma adenosine concentrations and SBP when data for DS and DR were analyzed together ($r = 0.34$, $p < 0.05$, fig. 2).

Discussion

Much evidence suggests that adenosine may be a physiological modulator of vascular smooth muscle tone, sympathetic neurotransmission, renin release, and renal and cardiac function. Along this line, considerable attention has been focussed on the possible role of adenosine in cardiovascular physiology and pathophysiology[6]. Previously, we developed a specific and sensitive assay for adenosine based on fluorescent determination of adenine compounds by HPLC and showed that plasma adenosine concentrations are significantly higher in 13-week-old SHR with hypertension than in age-matched WKY[5]. In this study, we measured plasma adenosine levels in rats with another type of genetic hypertension. Plasma adenosine levels were significantly higher in DS on a high salt diet than in DR on either a high salt or a low salt diet, and a significant positive correlation was found between plasma adenosine levels and SBP when data from DS and DR were analyzed together. The salt content in the diet did not affect plasma adenosine levels in either DS or DR.

Little is known about the role of endogenous adenosine in the regulation of blood pressure and sodium balance. The actions of adenosine are mediated by cell surface receptors. There are at least two different subtypes of adenosine receptors, which are known as A1 and A2 receptors, and the effect of adenosine depends on the distribution of these receptor subtypes[7]. Intravenous administration of adenosine lowers blood pressure in most animal models[8]. This action can be explained by the A2-receptor-mediated vasodilatory and A1-receptor-mediated neuroinhibitory effects of adenosine. In contrast, adenosine produces vasoconstriction in the kidney[3]. Renal vasoconstriction is mediated by A1 receptors and involves an interaction with angiotensin II[9]. In the isolated perfused rat kidney model, stimulation of A1-receptors inhibited the excretion of sodium, and stimulation of A2 receptors stimulated it[10].

It is most likely that adenosine increased in the circulation of DS on a high salt diet as a result of compensation for hypertension. However, the exact origin of the elevated plasma level remains to be determined. Sedaa et al. have shown that endothelial cells, nerve and smooth muscle cells are the sites involved in the nerve stimulation and α-agonist-induced overflow of endogenous adenine nucleosides and nucleotides from rabbit aorta, and that the removal of endothelium reduces the release of adenosine and adenine nucleotides by 93%[11]. From these studies it seems likely that a significant portion of the circulating adenosine is derived from endothelium.

On the other hand, adenosine stimulates afferent fibres mediating sympathetic activity, including renal and myocardial afferent nerves, and carotid and aortic chemoreceptors[12–15]. Katholi et al. have reported that adenosine produces hypertension due to the activation of renal afferent nerves, when it is infused into the renal artery in some animal models[16]. In this context, increased renal afferent nerve activity might be related to elevated blood pressure in DS on a high salt diet. Recently, FK453, a novel A1 receptor antagonist, has been shown to have natriuretic and hypotensive effects in patients with essential hypertension[17]. It seems worthwhile evaluating whether A1 receptor activity plays a role in the regulation of blood pressure and renal sodium handling in DS on a high salt diet.

In conclusion, plasma adenosine levels are increased in DS on a high salt diet and may be at least partly involved in the pathophysiology of high blood pressure in this model of hypertension. Further investigations are needed to elucidate the pathophysiological significance of increased plasma adenosine levels in DS.

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