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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Adenosine, an Identified Active Component from the Driselase-Treated Fraction of Rice Bran, Is Effective at Improving Metabolic Syndrome in Stroke-Prone Spontaneously Hypertensive Rats

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In the present study, we isolated and identified an active component from the Driselase-treated fraction and investigated its effect by acute and chronic oral administration on hypertension, lipid, and glucose metabolism in stroke-prone spontaneously hypertensive rats. The active component was identified as adenosine and improves hypertension after single oral administration. Rats who were 10 weeks old were divided into control and adenosine groups and were administered water or water with adenosine (10 mg/L), respectively, for 3 weeks. Hypertension and plasma lipid, nitric oxide, insulin, leptin, adiponectin levels, and glucose metabolism were significantly improved in the adenosine group. The mRNA expression levels of genes involved in lipid and glucose metabolism were altered in the adenosine group. Single oral administration of adenosine (10 mg/kg body weight) improved hypertension and plasma triglyceride, glucose, and nitric oxide levels 2 h after administration. In conclusion, oral acute and chronic administration of adenosine are beneficial and improve the metabolic syndrome-related disease parameters.

KEYWORDS: Adenosine; rice bran; hypertension; lipid profile; glucose metabolism; hepatic gene expression

INTRODUCTION

Multiple factors related to the metabolic state, such as changes in plasma glucose, lipid profiles, and insulin resistance, are involved in the management of hypertension. With increasing knowledge of the link between diet and health, there is an emerging interest in the use of naturally active compounds such as functional food ingredients and nutraceuticals in therapeutic applications. The consumption of various edible plants, vegetables, fruits, and cereals has been regarded as a factor that could prevent these chronic diseases. Therefore, any active compounds from food having the ability to improve hypertension are potential candidates for the prevention or improvement of these diseases.

Rice bran is a byproduct of the rice milling process, and it contains various active compounds that impart beneficial effects on human health. It is well-known that the major active compounds of rice bran are derived from rice bran oil. From the results of human and animal studies, researchers have demonstrated that the hypocholesterolemic effect of rice bran oil is attributable to its specific components, such as γ-oryzanol and γ-tocotrienol (*1*−*4*). Rice bran also contains a high level of dietary fibers (β-glucan, pectin, and gum) and ferulic acid (*5, 6*). Recently, we determined that the Driselase-treated fraction (DF) of rice bran—the filtrate of defatted rice bran treated with plant cell wall-degrading enzyme—has a beneficial effect on lowering hypertension and improving lipid profiles (*7*); when compared to ferulic acid as a purified component of plant cell walls, the DF was more effective in improving hypertension and glucose and lipid metabolisms in stroke-prone spontaneously hypertensive rats (SHRSP) (*8*).

In this study, we developed a fractionation method for the identification of active components contained in the DF of rice bran, and we found that adenosine was one of the active components and that it improved hypertension after oral administration. Various studies have indicated that adenosine has health-related physiological activities; for example, continuous intravenous adenosine infusion in premature babies has dramatic effects in therapy for neonatal refractory pulmonary

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hypertension (*9*), and adenosine attenuates proliferation in both human and rat glomerular mesangial cells associated with hypertension and diabetes (*10*). Adenosine mediates its effects by activating specific subtypes of adenosine receptors, which exist in multiple subtypes (A_1, A_{2A}, A_{2B}, and A_3) (*11, 12*). Recently, adenosine was also found to decrease blood glucose and insulin concentrations in rat and human studies. This was observed after adenosine was orally administered in the presence of sucrose solution due to the inhibitory activity of α-glucosidase (*13, 14*).

On the basis of this background, the purpose of the present work is to establish whether orally acute or chronic adenosine administration has physiological effects on the improvement of hypertension, hyperlipidemia, and hyperglycemia in SHRSP, an animal model of hypertension-related disorders essentially similar to hypertension and hyperlipidemia in humans.

MATERIALS AND METHODS

Extraction, Isolation, and Identification of Active Components from the DF of Rice Bran. The DF was prepared based on the method used in our previous study (*7*). Briefly, rice bran (500 g) was agitated in 1.0 L of 70% ethanol for 2 h and filtered, yielding two fractions (solid and suspension fractions). The DF was derived from the solid fraction. The solid fraction was dried at room temperature and then resuspended in 500 mL of 10 mM acetate buffer (pH 5.0) containing Driselase (0.2 g/L, Sigma Chemical Co., St. Louis, MO). After Driselase treatment overnight at 37 °C, the suspension was filtered and finally lyophilized. Column chromatography was conducted on silica gel 60 (Kanto Chemical, Japan) and ODS (Fuji Silysia, Japan). Thin-layer chromatography was carried out using precoated silica gel plates (Merck, Japan), and spots were detected by spraying with 10% vanillin in H_2SO_4, followed by heating, or by UV irradiation. The DF (14.3 g) was chromatographed on a silica gel column and eluted with a *n*-hexane/ethyl acetate stepwise gradient (0−100), ethanol/methanol (50:50), and methanol (100). Fraction 1 was obtained from *n*-hexane/ethanol (30:70−40:60) eluates. Ethanol/methanol (50:50) and methanol (100) eluates were further purified by ODS column chromatography and eluted with a water/methanol stepwise gradient elution. Fractions 2 and 3 derived from ethanol/methanol (50:50) eluates were obtained from water/methanol (90:10−60:40) eluates and water/methanol (30:70−0:100) eluates, respectively, by using an ODS column. Fraction 4 derived from methanol (100) eluate was obtained from water/methanol (80:20−30:70) eluates by using an ODS column. We observed the blood pressure (BP)-lowering activity of each fraction by single oral administration to SHRSP. Each fraction dissolved in distilled water was administered orally to SHRSP (10 weeks old) via a gastric tube (40 mg/kg body weight). The BP was measured before and 1, 2, 4, and 6 h after the administration. We found fraction 4 to be the best to reduce the BP 1, 2, 4, and 6 h after the administration ($p < 0.05$) (data not shown). Therefore, we separated fraction 4 (3.68 g) by high-performance liquid chromatography (HPLC) using an ODS column. HPLC was conducted in a column chromatorex-ODS (i.d. 10 mm × 20 mm, Fuji-Division Chemical, Co., Ltd., Japan) with a detection limit of 280 nm. A linear gradient was used, and the mobile phase was changed from 50 mM acetate buffer (pH 4.0) in water/acetonitrile (95:5) to 50 mM acetate buffer (pH 4.0) in water/acetonitrile (35:65) in 40 min at a flow rate of 1.0 mL/min. We found that fractions 5 (215 mg), 6 (112 mg), 7 (102 mg), 8 (75.1 mg), 9 (77.5 mg), and 10 (70.2 mg) were obtained at retention times of 16, 17, 18, 21, 22, and 24−25 min, respectively. The percentage weight by weight recovery of each fraction was 5.84, 3.03, 2.77, 2.04, 2.11, and 1.91, respectively. We examined the BP-lowering activity of each fraction by single oral administration to SHRSP (14 weeks old). We found fraction 9 to be the best to reduce the BP 1, 2, 4, and 6 h after the administration ($p < 0.05$) (data not shown). Because the oral administration of fraction 9 at 40 mg/kg body weight decreased the BP significantly 1, 2, 4, and 6 h after administration, we performed an additional experiment on 12 week old rats to verify these data. At 12 week, the rats were divided into two groups ($n = 4$): one for the control group and the other for the fraction 9 group.

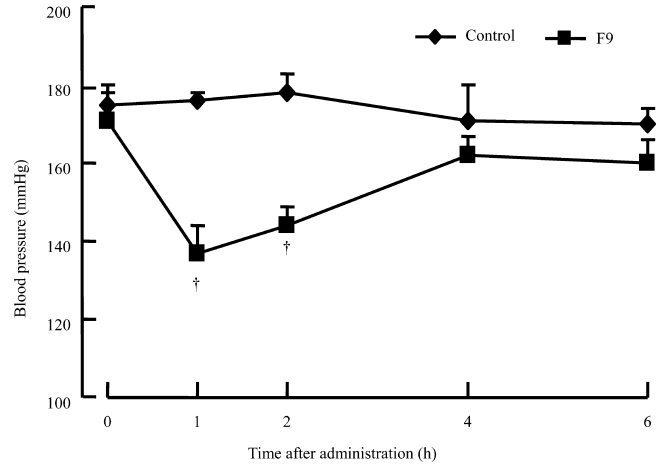

Figure 1. Effect of fraction 9 (F9) administration (40 mg/kg body weight) by single injection on systolic BP in rats. Values are means ± SEMs, $n = 4$; †significant ($p < 0.01$).

SHRSP were fasted for 16 h and then orally administered fraction 9 after it was dissolved in distilled water at 40 mg/kg body weight or distilled water alone as a control (1 mL) via a gastric tube. The systolic BP was measured before and 1, 2, 4, and 6 h after the administration. When compared to the control group, we observed a hypotensive effect 1 and 2 h after the administration ($p < 0.01$), and the basal condition was restored after 4 and 6 h (**Figure 1**). Furthermore, we attempted to determine the chemical structure of this fraction (fraction 9) by fast atom bombardment−mass spectrometry (FAB-MS) and NMR analyses. Mass spectra were obtained using a JEOL JMS-700 instrument, and ^1H and ^{13}C NMR spectra were acquired using a JEOL JNM-EX-400 spectrometer. Chemical shifts were obtained on a δ (ppm) scale with tetramethylsilane as an internal standard. Finally, we identified the active compound as adenosine. In view of the result that adenosine caused a decrease in the BP after single oral administration, we used pure adenosine compound (Wako Pure Chemical Co., Osaka, Japan) for further animal experiments (acute and chronic oral administration).

Animal Experiments and Diet. Male SHRSP, Izumo strain (Japan SLC, Shizuoka, Japan), were used in these studies. The rats were housed in individual stainless steel cages under a controlled atmosphere (temperature, 23 ± 2 °C; humidity, 50 ± 10%; 12 h light−dark cycle). The rats were given free access to a set diet (F-2, Funabashi Farm, Chiba, Japan) and drinking water. At the beginning of both studies, we used animals with the same body weight. In the acute experiment, we categorized animals with the same body weight into two groups before oral administration. The experimental plan for the present study was approved by the Animal Research-Animal Care Committee of the Graduate School of Agricultural Science, Tohoku University. The entire experiment was conducted in accordance with the guidelines issued by this committee and the Japanese governmental legislation (1980). The same committee supervised the care and use of the rats used in the present study.

BP Measurements. The BP was measured using the tail cuff method with a BP meter without warming (MK-2000, Muromachi Kikai, Tokyo, Japan), as described previously (*7*). A minimum of six BP measurements were obtained for each rat. The average value of four consistent readings of the systolic BP was regarded as the individual systolic BP.

Acute Administration of Adenosine. After an 1 week acclimatization period, 13 week old rats were divided into two groups: One group was the control, and the other was the adenosine group ($n = 4$). After 16 h of fasting, adenosine dissolved in distilled water was administered orally to SHRSP (10 mg/kg body weight), and distilled water was administered in the control group (1 mL) via a gastric tube. The BP was measured before and 1, 2, 4, and 6 h after the administration. One week later, the rats were administered adenosine again by the same procedure and sacrificed after 2 h of oral administration under light diethyl ether anesthesia. Blood was collected from the abdominal aorta, and plasma was separated for later analysis. Dissected livers were frozen at −80 °C until later analysis.

Table 1. Sequences of Primers for PCR Amplification

gene[a]	forward primer	reverse primer
EF-1	GATGGCCCCAAATTCTTGAAG	GGACCATGTCAACAATTGCAG
FASN	GGCTCACACACCTACGTATTGG	TGCTTAATGAAGAAGCATATGGCTT
PPARγ	GGCCATATTTATAGCTGTCATTA TTCTC	GCAGCAGGTTGTCTTGGATGT
SREBP1c	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGCCCAG
ACC	TTGTGGAAGTGGAAGGCACAG	CCTTATTATTGTCCCAGACGTAAGC
eNOS	GACCCTGCCAACGTGGAG	GGTCCAGCCATGTTGAATACAGA
CPT1	GGTGGAGCTCTTTGACTTTGAGAA	GGCTAGAGAACTTGGAAGAAATATGG
SOD	GGCCGTACTATGGTGGTCCA	TCCACCTTTGCCCAAGTCAT
GSHPx	TGACCGACCCCAAGTACATCA	AAATGTCGTTGCGGGACAC
PPARα	TGTGGCTGCTATAATTTGCTGTG	CTCCTGCAACTTCTCAATGTATCCT
ACOX	TTCGTCCAGCCAGATTGGT	CGAACAAGGTCGACAGAGGTTAG
VLACS	ACTCTTTCAGCACATCTCGGAGTA	CCGGTCATCTCAATGGTATCTTGTA
LFABP	AGTACCAAGTGCAGAGCCAAGAG	GCAGACCCATCGCCTTCA
G6Pase	TTGTGCATTTGCTAGGAAGAGAAG	ATCTAAAGACCCAGGCATAACTGAAG

[a] EF-1, eukaryotic elongation factor-1α1; FASN, fatty acid synthase; PPARγ, peroxisome proliferator-activated receptor γ; SREBP1c, sterol regulatory element-binding protein-1c, ACC, acetyl-CoA carboxylase α; eNOS, endothelial nitric oxide synthase; CPT1, carnitine palmitoyltransferase 1; SOD, superoxide dismutase; GSHPx, glutathione peroxidase; PPARα, peroxisome proliferator-activated receptor α; ACOX, acyl-CoA oxidase; VLACS, very long chain acyl-CoA synthetase; LFABP, liver fatty acid binding protein; G6Pase, glucose-6-phosphatase.

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Chronic Administration of Adenosine. After an 1 week acclimatization period, 10 week old SHRSP were divided into two groups: One group was the control, and the second was the adenosine group based on body weight ($n = 4$). The control and adenosine groups were administered distilled water and distilled water containing 10 mg/L adenosine, respectively, for 3 weeks. Water and food intake were recorded every day. The systolic BP and body weight were measured every week during experimental periods. Systolic BP was measured during the day time (02.00−04.00 pm). At the end of the experimental period, the rats were sacrificed under light diethyl ether anesthesia after 16 h of fasting. Blood was collected from the abdominal aorta, and the plasma was immediately separated by centrifugation and stored at −20 °C until analysis. Liver and epididymal fat were excised and weighed. Livers were frozen at −80 °C until later analysis.

Oral Glucose Tolerance Test (OGTT). Ten week old of SHRSP were used for an OGTT. The animals were fasted for 16 h, and blood for glucose and insulin measurement was collected from the tail vein before (0) and 30, 60, and 120 min after administration of 1 mL of 20% glucose solution as the control ($n = 4$) and 1 mL of 20% glucose containing 40 mg/mL adenosine ($n = 4$) via a gastric tube. Plasma glucose levels were measured by enzymatic colorimetric methods (Wako Pure Chemical Co.). Plasma insulin levels were measured by using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit from Shibayagi (Shibayagi Co., Gunma, Japan). The analysis of the incremental area under the curve (iAUC) of the plasma glucose and insulin response was calculated based on the method of Wolever and Jenkins (*15*).

Plasma and Liver Parameters. Plasma levels of blood urea nitrogen (BUN), creatinine, albumin, glucose, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic colorimetric methods (Wako Pure Chemical Co.). Plasma leptin levels were measured by using a rat leptin ELISA kit from Yanaihara (Yanaihara Inst. Inc., Shizuoka, Japan). Plasma adiponectin levels were measured by using a rat adiponectin ELISA kit from Otsuka (Otsuka Co., Tokyo, Japan). The nitric oxide (NO) level in the plasma was quantified by the Griess method [NO_2/NO_3 Assay kit-C II (Colorimetric) Dojindo, Kumamoto, Japan], as described previously (*8*). The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula (*16*). Liver total lipids were determined by the Folch method (*17*). Liver TC and TG concentrations were determined using the same kit as that used for plasma TC and TG, following the extraction of liver samples with methanol−chloroform (1:2, v/v).

RNA Preparation and Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). Total RNA was isolated from the liver with a guanidine isothiocyanate-based reagent, Isogen (Nippon Gene, Japan), according to the instruction manual. The measurement of the wavelength ratio at 260 and 280 nm and agarose gel electrophoresis were performed to facilitate the quantitative and qualitative analysis of the isolated RNA. Five micrograms of total RNA was used as a template to synthesize the cDNA. The RNA was denaturated with oligo-dT/random primers; 10 mmol/L dNTP (Amersham Biosciences) and distilled water were added at 65 °C for 5 min. The RNA was then incubated in 50 mmol/L Tris-HCl buffer (pH 8.3); 0.1 mol/L DTT containing 50 units of SuperScript III reverse transcriptase (Invitrogen) and 20 units of RNaseOUT RNase inhibitor (Invitrogen) were added in 20 μL at 25 °C for 5 min, 50 °C for 60 min, and 70 °C for 15 min. Aliquots of the cDNA were used as a template for the subsequent quantitative RT-PCR using an Applied Biosystems Sequence Detection System 7300 (Foster City, CA) and SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. The relative gene expression levels were normalized by the amount of eukaryotic elongation factor 1α1 (EF1) mRNA (*18*). The genes listed in **Table 1** were amplified by cDNA specific primers.

Data Analysis. Values are presented as means ± standard errors of the mean (SEMs). The differences between group means were evaluated by Student's *t* test (SPSS statistical package, version 11.0). Differences were considered significant at $p < 0.05$.

RESULTS

Growth Parameter. There was no difference in the final body weight, body weight gain, food intake, water intake, and relative liver weight between the two groups in the chronic administration experiment (data not shown); however, the relative epididymal fat weight was significantly lower in the adenosine group (1.1 ± 0.1 g/100 g body weight) as compared to the control group (1.4 ± 0.1 g/100 g body weight) ($p < 0.05$). The mean daily intake of adenosine was 0.31 ± 0.02 mg/day during the experimental period.

BP. A hypotensive effect was observed after single oral administration at 1, 2, and 4 h; furthermore, after 6 h of administration, the basal condition was restored (**Figure 2A**). The reduction in BP was approximately 46, 24, and 25 mmHg after 1, 2, and 4 h of the administration, respectively. A BP-lowering activity was also observed in the chronic administration of adenosine. The systolic BP in the second and third week after the start of adenosine solution intake was significantly lower than that in the control group ($p < 0.01$) (**Figure 3A**).

Blood Parameter. Table 2 summarizes the parameters of the lipid profile, glucose level, and kidney functions after single oral administration of adenosine. The adenosine group revealed significantly lower plasma TG and glucose levels than the control group. **Table 3** shows the plasma and liver biochemical parameters in the chronic administration experiment. After 3 weeks of adenosine solution intake, rats showed a significant decrease in plasma TC, HDL-C, LDL-C, TG, FFA, BUN, albumin, hepatic TC, and hepatic TG levels as compared to the control group. Furthermore, the values of plasma glucose ($p < 0.01$) and insulin ($p < 0.05$) levels in the adenosine group were significantly lower than those in the control (**Table 3**). Next, we also examined the effect of adenosine administration at a dose of 40 mg/mL in 20% glucose solution on OGTT in rats (**Figures 4A** and **5A**). The blood glucose and insulin levels decreased 30 and 60 min after adenosine administration, respectively, as compared with the values in the control group. Moreover, the iAUCs for plasma glucose and insulin concentrations were significantly less than those for the control group (**Figures 4B** and **5B**).

The adenosine group exhibited a significant reduction in the plasma leptin and increase in the plasma adiponectin levels as compared to the control group ($p < 0.05$) (**Table 4**). Further-

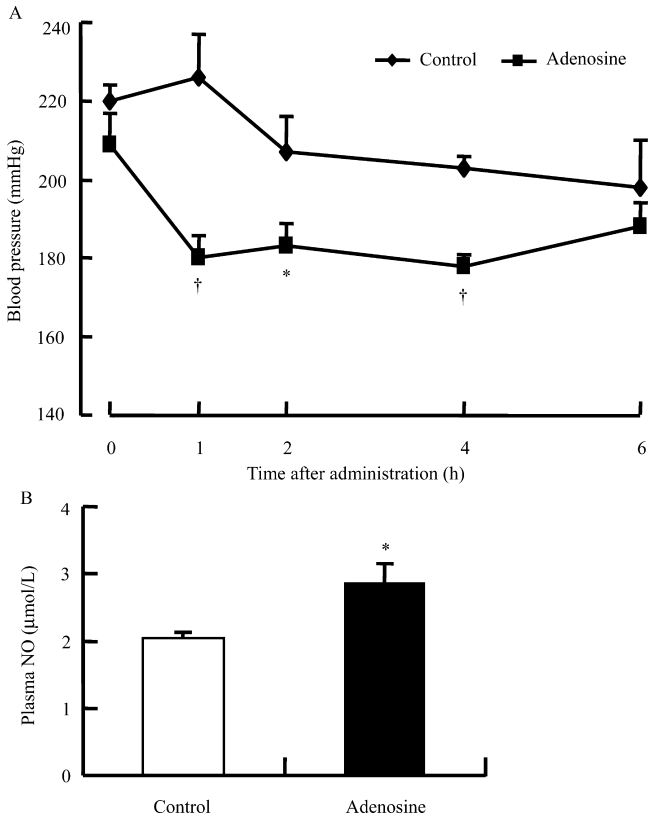

Figure 2. Effect of adenosine administration (10 mg/kg body weight) by single oral administration on systolic BP (**A**) and plasma NO (**B**) in rats. Values are means ± SEMs, $n = 4$; *significantly different from the control group ($p < 0.05$); †significantly different from the control group ($p < 0.01$).

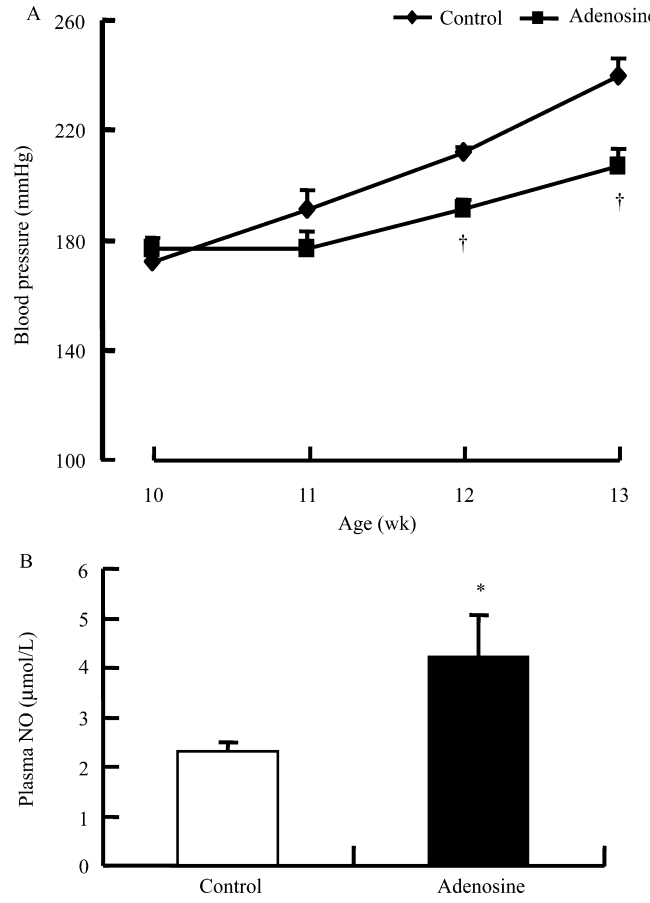

Figure 3. Effect of adenosine administration by chronic administration on the systolic BP (**A**) and plasma NO (**B**) in rats. Values are means ± SEMs, $n = 4$; *significantly different from the control group ($p < 0.05$); †$p < 0.01$.

more, the plasma NO levels of adenosine in both experimental studies were significantly higher ($p < 0.05$) when compared with those in the control group (**Figures 2B** and **3B**).

Hepatic Gene Expression Levels. We investigated the effect of adenosine with either single dose or chronic administration on hepatic mRNA expression by the quantitative RT-PCR method. As compared to the control group, the adenosine group showed a significant downregulation of hepatic mRNA levels of peroxisome proliferator activated receptor-γ (PPARγ) and liver fatty acid binding protein (LFABP) in the single dose adenosine administration experiment ($p < 0.05$) (**Table 5**). In the chronic administration experiment, the adenosine group exhibited significantly downregulated expression levels of PPARγ, glucose-6-phosphatase (G6Pase), and LFABP and upregulated expression levels of endothelial nitric oxide synthase (eNOS), carnitine palmitoyltransferase 1 (CPT1), and acyl-CoA oxidase (ACOX) (**Table 6**).

Table 2. Plasma and Liver Biochemical ParAmeters after 2 h of Adenosine Administration[a]

biochemical parameter	control	adenosine
	plasma	
TC (mmol/L)	1.80 ± 0.06	1.78 ± 0.03
HDL-C (mmol/L)	1.00 ± 0.01	1.01 ± 0.05
LDL-C (mmol/L)	0.60 ± 0.05	0.64 ± 0.03
TG (mmol/L)	1.01 ± 0.05	0.65 ± 0.09†
FFA (mequiv/L)	0.36 ± 0.04	0.31 ± 0.01
glucose (mmol/L)	9.46 ± 0.50	8.20 ± 0.10*
BUN (mmol/L)	5.87 ± 0.38	5.70 ± 0.46
creatinine (μmol/L)	77.06 ± 3.25	74.23 ± 2.64
albumin (μmol/L)	0.56 ± 0.01	0.55 ± 0.01
	liver	
total lipid (mg/g)	55.03 ± 2.19	53.67 ± 1.37
TC (mg/g)	8.74 ± 1.03	9.83 ± 1.53
TG (mg/g)	16.13 ± 1.40	16.22 ± 2.14

[a] Values are means ± SEM, $n = 4$. *Significantly different from the control group ($p < 0.05$); †$p < 0.01$.

DISCUSSION

The results of this study demonstrated that short- and long-term oral ingestion of adenosine have numerous protective effects to improve hypertension, hyperlipidemia, and hyperglycemia in SHRSP as compared to the control group. This is the first report regarding the potential therapeutic effects of orally administered adenosine in spontaneous hypertensive conditions using SHRSP as an animal model of hyperlipidemia (*19*), severe hypertension, and multisystem end-organ damage with prominent involvement of the kidney (proteinuria) (*20*).

It is well-established that NO produced by endothelial cells plays a pivotal role in the maintenance of vascular function (*21*) by elevating the intracellular cyclic GMP levels and causing smooth muscle relaxation (*22*). Several studies have reported that the impaired NO release from the endothelial cells (*23*) and kidneys (*24*) is related to the high BP in SHRSP. In the present studies, we observed that treatment with adenosine in both single and chronic administration showed increased plasma NO levels (**Figures 2B** and **3B**), and these results corresponded well with the hypotensive effect observed in SHRSP (**Figures 2A** and **3A**). The increases in plasma NO levels were a consequence of the vasodilation effect of adenosine administra-

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Table 3. Plasma and Liver Biochemical Parameters after Chronic Adenosine Administration[a]

biochemical parameters	control	adenosine
	plasma	
TC (mmol/L)	1.49 ± 001	1.39 ± 0.01*
HDL-C (mmol/L)	0.81 ± 0.04	0.93 ± 0.03*
LDL-C (mmol/L)	0.54 ± 0.05	0.34 ± 0.03*
TG (mmol/L)	0.73 ± 0.02	0.58 ± 0.05*
FFA (mequiv/L)	0.33 ± 0.02	0.26 ± 0.01†
BUN (mmol/L)	5.44 ± 0.28	4.46 ± 0.32*
creatinine (μmol/L)	67.07 ± 0.24	62.19 ± 4.09
BUN/creatinine	20.04 ± 2.20	17.9 ± 1.50
albumin (μmol/L)	0.57 ± 0.02	0.52 ± 0.01*
glucose (mmol/L)	9.18 ± 0.28	7.53 ± 0.14†
insulin (ng/mL)	3.32 ± 0.29	2.38 ± 0.15*
	liver	
total lipid (mg/g)	58.24 ± 4.14	55.63 ± 5.26
TC (mg/g)	14.31 ± 0.59	8.62 ± 1.08†
TG (mg/g)	22.63 ± 1.74	14.54 ± 2.97*

[a] Values are means ± SEM, $n = 4$. *Significantly different from the control group ($p < 0.05$); †$p < 0.01$.

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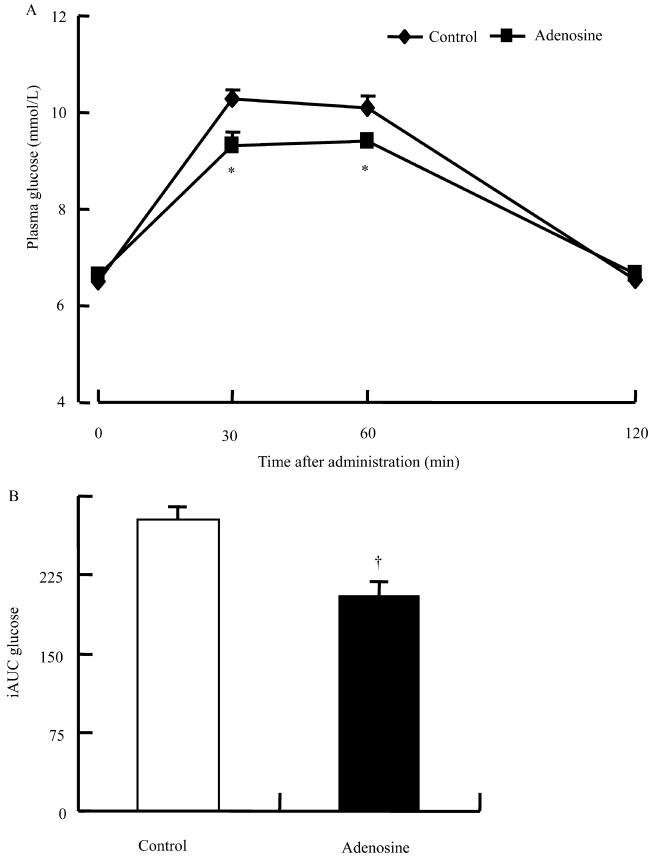

Figure 4. Changes in plasma glucose (**A**) and iAUC (**B**) of the OGTT in rats. Values are means ± SEMs, $n = 4$; *significantly different from the control group ($p < 0.05$); †$p < 0.01$.

tion. The hypotensive effect of adenosine could be due to the fact that adenosine causes potent vasodilation by acting at adenosine receptors (A_2) on vascular smooth muscles (*25*), and it may also stimulate K^+_{ATP} channels, resulting in the hyperpolarization of smooth muscles (*26*). We also observed that adenosine treatment with chronic administration significantly ameliorates the parameters of kidney function, such as plasma BUN and albumin (**Table 3**); this result is consistent with a previous report showing that adenosine attenuation of proliferation in both human and rat glomerular mesangial cells is associated with hypertension and diabetes (*12*). We believe that

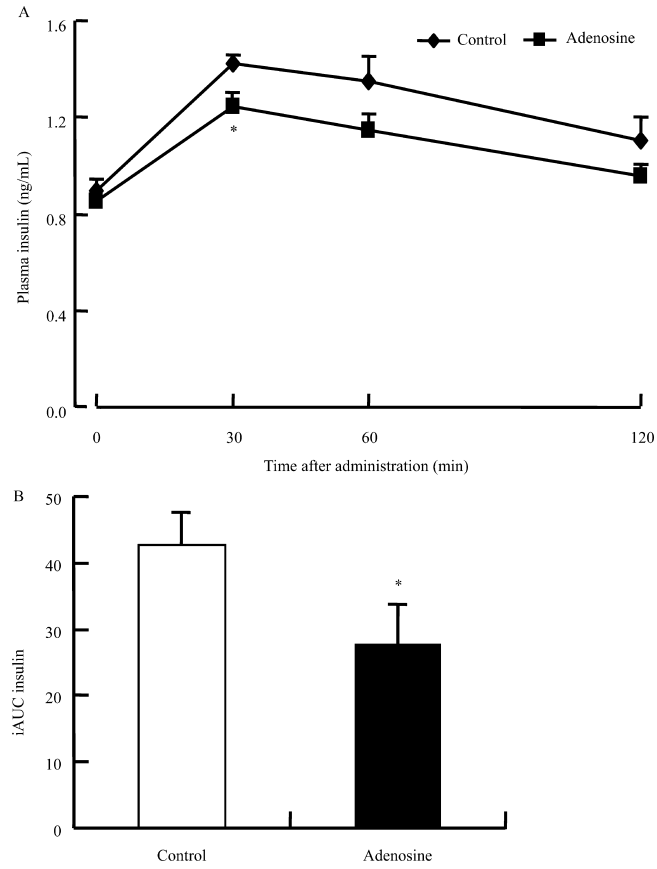

Figure 5. Changes in plasma insulin (**A**) and iAUC (**B**) of the OGTT in rats. Values are means ± SEMs, $n = 4$; *significantly different from the control group ($p < 0.05$); †$p < 0.01$.

Table 4. Plasma Leptin and Adiponectin with Chronic Adenosine Administration[a]

variable	control	adenosine
leptin (ng/mL)	2.43 ± 0.15	2.08 ± 0.05*
adiponectin (μg/mL)	9.55 ± 0.60	10.95 ± 0.43*

[a] Values are means ± SEM, $n = 4$. *Significantly different from the control group ($p < 0.05$).

Table 5. Hepatic mRNA Expression Level by Quantitative RT-PCR[a] after 2 h of Adenosine Administration[a]

gene	control	adenosine
PPARγ	1.0 ± 0.1	0.7 ± 0.1*
SREBP1c	1.0 ± 0.1	0.9 ± 0.2
G6Pase	1.0 ± 0.1	0.8 ± 0.2
LFABP	1.0 ± 0.2	0.5 ± 0.2*

[a] Expression of mRNA (fold). Values are means ± SEMs, $n = 4$. *Significantly different from the control group ($p < 0.05$).

adenosine administration may improve the depressed effect on vasodilation in the SHRSP; however, further investigation is required for elucidating the actual mechanism responsible for this phenomenon.

The expression of eNOS plays a critical role in maintaining basic vasotonus in the hemodynamic regulation by NO in hypertension, thrombosis, or atherosclerosis (*27*). Our present result showed that the chronic administration of adenosine results in significant upregulation of the hepatic mRNA level of eNOS (**Table 6**); therefore, we supposed that the upregulation of this gene corresponded to the increase in the plasma NO level. Furthermore, we observed that the mRNA expression level of

Table 6. Hepatic mRNA Expression Level by Quantitative RT-PCR for Chronic Adenosine Administration[a]

gene[b]	control	adenosine
PPARγ	1.0 ± 0.2	0.6 ± 0.2*
SREBP1c	1.0 ± 0.2	0.8 ± 0.2
G6Pase	1.0 ± 0.1	0.6 ± 0.1*
FASN	1.0 ± 0.2	1.5 ± 0.3
ACC	1.0 ± 0.3	1.3 ± 0.7
eNOS	1.0 ± 0.6	4.1 ± 1.0*
SOD	1.0 ± 0.2	1.2 ± 0.2
GSHPx	1.0 ± 0.1	1.1 ± 0.1
PPARα	1.0 ± 0.3	1.5 ± 0.4
LFABP	1.0 ± 0.2	0.4 ± 0.2*
CPT1	1.0 ± 0.3	3.3 ± 1.0*
ACOX	1.0 ± 0.3	2.8 ± 0.6*
VLACS	1.0 ± 0.2	0.9 ± 0.3

[a] Expression of mRNA (fold). Values are means ± SEMs, $n = 4$. *Significantly different from the control group ($p < 0.05$). [b] ACC, acetyl-CoA carboxylase α.

the gene related to antioxidative activity, such as SOD, tended to increase (**Table 6**). It is well-established that NO produced by endothelial cells plays a pivotal role in the maintenance of vascular function and health (*28*) by elevating the intracellular cyclic GMP levels and causing smooth muscle relaxation (*29*). Despite the rapid growth in the number of NO experiments, the compound still remains to be clearly understood. In fact, there are many contradictory reports on NO. For example, NO has been found to cause tissue damage in ischemia-reperfusion systems; however, there is also evidence that NO is generated to protect tissues from such damage. We supposed that the upregulation of the eNOS mRNA level in the liver contributes to improve oxidative damage; this inhibits the progression of hypertension and hyperlipidemia in SHRSP.

Current studies have shown that adipose tissue not only stores excess energy in the form of fat but also secretes physiologically active substances called adipocytokines (*30*). SHRSP is inherently insulin resistant (*19*); leptin also accumulates in the plasma to lead to the development of hypertension (*31*). In the present study, we showed that a significant decrease in plasma leptin corresponded to the decreasing relative epididymal fat weight. Because several reports indicate that adiponectin can lead to enhanced insulin action in vitro and in vivo, it is strongly suggested that adiponectin plays a protective role against insulin resistance (*32*−*34*). This result is consistent with a previous report showing that thiazolidinediones (TZDs), synthetic insulin sensitizers, increased the plasma adiponectin levels in C57BL/KsJ obese and diabetic mice and the secretion of adiponectin in 3T3-L1 adipocytes (*35*). Furthermore, it was reported that hypoadiponectinemia is associated with endothelial dysfunction and causes diet-induced hypertension (*36*). These results suggest that an enhanced plasma adiponectin level alleviates hyperinsulinemia (**Table 4**) and may prevent the development of hypertension in SHRSP. We speculated that the ability of dietary adenosine to enhance the plasma adiponectin level might be caused by the downregulation of PPARγ mRNA after adenosine treatment.

In the present study, we found that both single oral administration and chronic administration of adenosine profoundly decreased hepatic mRNA levels of PPARγ, G6Pase, and LFABP (**Tables 5** and **6**). The mRNA of PPARγ has a high expression level in adipose tissue, liver, macrophage, intestine, kidney, and muscle (*37*). PPARγ is also the molecular target of TZDs, which is an insulin-sensitizing drug used clinically in the treatment of type 2 diabetes (*38*). We suggested that adenosine administration can improve insulin sensitivity in the SHRSP (**Figures 4** and **5**). To date, the detailed mechanism regarding the effect of adenosine on the glucose metabolism in this study remains unclear, due to inhibited glucose absorption in the intestine or stimulated glucose transport in the liver; future studies are required to determine the detailed mechanism. Our data showed that adenosine improved the plasma glucose level in both experiments (**Tables 2** and **3**); moreover, our data exhibited that the hepatic mRNA expression level of G6Pase, a gene-encoding rate-controlling enzyme of hepatic gluconeogenesis, was also downregulated 3 weeks after the ingestion of adenosine (**Table 5**). From the viewpoint of these studies, we suggest that adenosine can take part in improving the regulation of lipid and glucose metabolism in the liver.

In conclusion, our results indicated that acute oral administration and chronic administration of adenosine have novel functions in the BP-lowering effect in SHRSP. Furthermore, adenosine was found to improve hyperlipidemia and plasma kidney functions and reduce the plasma leptin level, thereby enhancing the action of insulin and the plasma adiponectin level accompanied by the alleviation of hyperinsulinemia. Adenosine also prevents the development of hypertension in SHRSP. Further studies should be performed to discover the complete absorption pathway of adenosine or its metabolites in rodents, and studies on the effects and safety concerns with regard to adenosine and its long-term administration are necessary.

ABBREVIATIONS USED

ACOX, acyl-CoA oxidase; BP, blood pressure; CPT, carnitine palmitoyltransferase; DF, Driselase-treated fraction; eNOS, endothelial nitric oxide synthase; EF1, eukaryotic elongation factor 1α1; FASN, fatty acid synthase; GSHPx, glutathione peroxidase; G6Pase, glucose-6-phosphatase; LFABP, liver fatty acid binding protein; PPARα, peroxisome proliferator-activated receptor-α; PPARγ, peroxisome proliferator-activated receptor-γ; SHRSP, stroke-prone spontaneously hypertensive rats; SOD, superoxide dismutase; SREBP1c, sterol regulatory element-binding protein-1c; VLACS, very long chain acyl-CoA synthetase.

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