

Antihypertensive Effect of Sesamin. III. Protection against Development and Maintenance of Hypertension in Stroke-Prone Spontaneously Hypertensive Rats

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The antihypertensive effect of sesamin, a lignan from sesame oil, was examined using salt-loaded and unloaded stroke-prone spontaneously hypertensive rats (SHRSP). The animals at 6 weeks of age were separated into a salt-loaded group and an unloaded group. Salt-loaded animals were maintained on 1% NaCl drinking water. Each group was further divided into two groups: normal-diet group and sesamin-diet group. Systolic blood pressure of all animals was monitored once weekly. At the end of the feeding periods, cardiovascular hypertrophy and renal damage were evaluated. In the salt-loaded group, sesamin feeding significantly suppressed the development of hypertension, and efficient suppression was maintained from 9 to 26 weeks (*e.g.*, 215 ± 4 vs. 180 ± 4 mmHg, at 17 weeks old). The left ventricle plus septum weight-to-body weight ratio was slightly but significantly lowered by sesamin feeding. When the degree of vascular hypertrophy of the aorta and superior mesenteric artery was histochemically evaluated, wall thickness and wall area of these vessels were significantly decreased by the sesamin feeding. Histological renal damage such as thickening of the tunica intima and fibroid degeneration of the arterial wall were often observed in the normal-diet group, but this damage was efficiently reduced in the sesamin-fed animals. On the other hand, in the salt-unloaded group, only a slight and non-significant suppressive effect of sesamin on the development of hypertension was observed. Although the wall area of the aorta was significantly decreased by the sesamin feeding, other vascular parameters were not ameliorated. The incidence of histological renal damage tended to decrease in sesamin-fed animals, but these alterations were not statistically significant. Thus, sesamin feeding was much more effective as an antihypertensive regimen in salt-loaded SHRSP than in unloaded SHRSP, thereby suggesting that sesamin is more useful as a prophylactic treatment in the malignant status of hypertension and/or hypertension followed by water and salt retention.

Key words sesamin; stroke-prone spontaneously hypertensive rat; hypertension; salt-loading; cardiovascular hypertrophy; renal damage

Sesamin is one of the lignans contained abundantly in sesame, but this lignan has not attracted nutritional or biological interest. Recently, several studies have focused on the biological activities of sesamin, and it was found that sesamin inhibits lipid metabolism such as desaturation in polyunsaturated fatty acid biosynthesis¹⁾ and cholesterol absorption.²⁾ Furthermore, sesamin is reported to exert protective effects against liver damage caused by alcohol or carbon tetrachloride,³⁾ and against 7,12-dimethylbenz[*a*]anthracene-induced rat mammary carcinogenesis.⁴⁾ These effects of sesamin may be due, at least in part, to immunopotential and antioxidative activity.⁴⁾ In addition, we noted that sesamin possesses Ca^{2+} -antagonistic vasorelaxing activity in the rat aortic ring (unpublished data). Previous studies have already demonstrated the Ca^{2+} -antagonistic activity of some lignans contained in Chinese herbs.⁵⁾ These findings led us to examine the possibility of sesamin acting as an antihypertensive treatment. In the first series, we demonstrated that dietary sesamin efficiently suppressed the development and maintenance of hypertension in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Both cardiac and vascular hypertrophies were also markedly suppressed by sesamin feeding.⁶⁾ Next, dietary sesamin ameliorated the development of hypertension and cardiac hypertrophy in two-kidney, one-clip (2K, 1C) renal hypertensive rats.⁷⁾ However, vascular hy-

pertrophy in this model was not suppressed by sesamin feeding, suggesting that sesamin is more effective in the vascular hypertrophy observed in salt-dependent hypertension. To further evaluate the antihypertensive activity of sesamin, we examined the effect of this lignan on the development of the hypertension of salt-loaded or unloaded stroke-prone spontaneously hypertensive rats (SHRSP). In this paper, we report that sesamin feeding in salt-loaded SHRSP efficiently suppresses the development of hypertension followed by cardiovascular hypertrophy and renal damage.

MATERIALS AND METHODS

Materials Sesamin was prepared from refined sesame oil and purified in our laboratory, as described previously.⁸⁾ Sesamin-containing diet (1 w/w% in commercial normal diet) was prepared at Oriental Yeast Co., Ltd. The concentration of sesamin was determined based on the previous study.³⁾ All other reagents used were of an analytical grade.

Animal Experiments Male SHRSP (6 weeks old, Kinki University School of Medicine, Osaka, Japan) were used. The rats were separated into a salt-loaded group and an unloaded group. In the salt-loaded group, 1% NaCl was added to their tap water to drink *ad libitum*. Salt-unloaded animals were given free access to tap water. Each group was further

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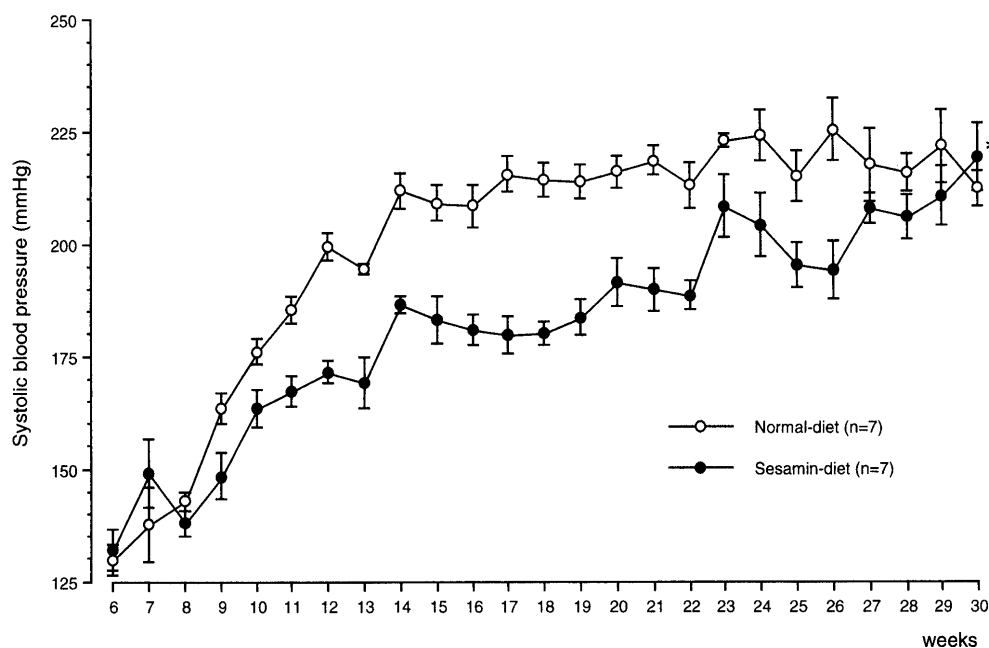


Fig. 1. Changes in Systolic Blood Pressure of Salt-Loaded SHRSP Fed Normal-Diet or Sesamin-Diet

Points and bars are the mean \pm S.E.M. * $p < 0.001$, compared with normal-diet group.

divided into two groups: normal-diet group and sesamin-diet group. Systolic blood pressure was monitored weekly with a tail cuff and a pneumatic pulse transducer (BP-98A, Softron). After 19 weeks (salt-unloaded group) or 24 weeks (salt-loaded group), the animals were killed by exsanguination under anesthesia (40 mg/kg sodium pentobarbital, i.p.), and the heart and left kidney were removed. Weights of the whole heart and the left ventricle plus the septum were determined. The thoracic aorta and superior mesenteric artery were also removed, from which fat and adherent connective tissue were cleaned off, and they were used for morphometric analysis.

Morphometric Analysis The thoracic aorta and superior mesenteric artery of each rat were placed in a vial of 10%-formaldehyde neutral buffer solution for analysis later. Cross sections from the thoracic aorta and mesenteric arteries were cut 5 μ m thick and stained with Elastica-van Gieson. The vessel wall area and thickness were determined in three to four different cross sections of each vessel using a computerized digitizing system (IBAS II, Carl Zeiss, Germany). The cross sectional area (wall area: S) of the vessels was calculated as: $S = \pi M(ED - M)$, where M is wall (media) thickness and ED is the external diameter. ED was calculated as: $ED = L_e / \pi$. M was calculated as: $M = (L_e - L_i) / 2\pi$. L_e and L_i are the total lengths of the adventitia and internal elastic membrane, respectively. The renal tissues were embedded in paraffin and cut into thin sections according to conventional techniques. The sections were stained with haematoxylin-eosin and periodic acid-Schiff. The severity of morphological damage was determined under a light microscope and scored using a blind protocol as follows: 0 none, 1 mild.

Statistical Analysis All values were expressed as the mean \pm S.E.M. Statistical analysis for sesamin feeding on blood pressure was performed using the two-way repeated ANOVA. Student's unpaired t -test was used for analyzing values (body weight, tissue weight, wall thickness and area of vessels) between the normal-diet and sesamin-diet groups. The data of histological renal damage were analyzed for sig-

nificant differences between the normal-diet and sesamin-diet groups using the Mann-Whitney-test and χ^2 -test. Differences were considered to have statistical significance at $p < 0.05$.

RESULTS

Effects of Dietary Sesamin on the Blood Pressure of Salt-Loaded SHRSP At the beginning of the experiment, the systolic blood pressure of salt-loaded normal-diet and sesamin-diet groups was 130 ± 4 and 132 ± 5 mmHg, respectively. As shown in Fig. 1, blood pressure was progressively elevated in both groups. However, the development of hypertension in salt-loaded SHRSP was efficiently suppressed by sesamin feeding ($p < 0.001$). At 12 weeks, the systolic blood pressure of the normal-diet and sesamin-diet groups was 199 ± 3 and 172 ± 2 mmHg, respectively. Efficient suppressive action of sesamin (15–30 mmHg decrease) was observed from 9 to 26 weeks during the feeding period. However, the blood pressure of both groups was at almost the same level at 30 weeks.

Effects of Dietary Sesamin on the Blood Pressure of Salt-Unloaded SHRSP At the beginning of the experiment, the systolic blood pressure of salt-unloaded normal-diet and sesamin-diet groups was 128 ± 2 and 125 ± 3 mmHg, respectively. As shown in Fig. 2, blood pressure was progressively elevated in both groups. The development and maintenance of the hypertension in SHRSP was slightly suppressed by the sesamin feeding, but this effect was not statistically significant.

Effects of Dietary Sesamin on Body Weights and Heart Weights of Salt-Loaded SHRSP The comparative data of body weights and heart weights in salt-loaded normal- and sesamin-diet groups are summarized in Table 1. A gain in body weight of the sesamin-diet group was smaller than that in the normal-diet group. Similarly, the heart weight and left ventricle (L.V.) + septum weight in the sesamin-diet group was significantly smaller than in the normal-diet group. Al-

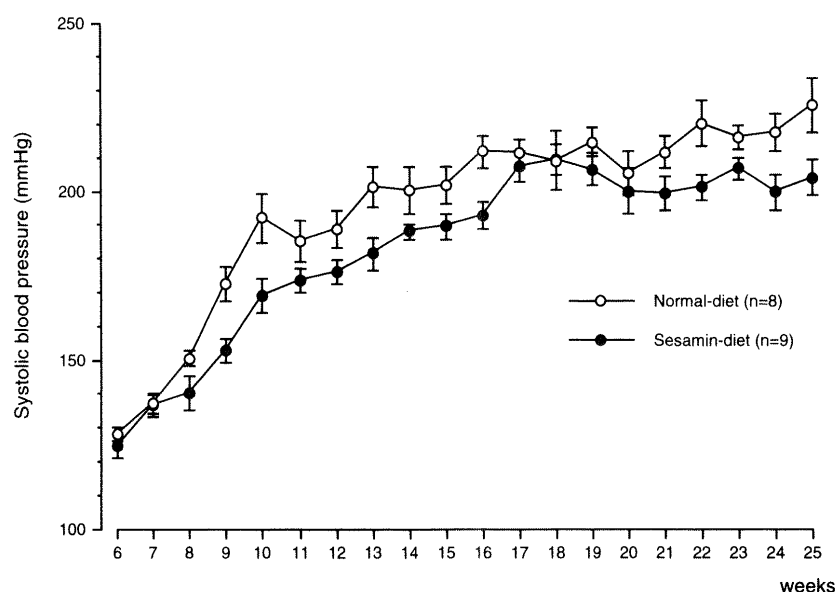


Fig. 2. Changes in Systolic Blood Pressure of Salt-Unloaded SHRSP Fed Normal-Diet or Sesamin-Diet

Points and bars are the mean \pm S.E.M.

Table 1. Comparative Data of Body Weights and Heart Weights in Normal- and Sesamin-Diet Groups

	Salt-loaded (1% NaCl)		Unloaded (H ₂ O)	
	Normal-diet (n=7)	Sesamin-diet (n=7)	Normal-diet (n=8)	Sesamin-diet (n=9)
Body weight (BW, g)	278 \pm 6	261 \pm 3*	287 \pm 6	246 \pm 8*
Heart weight (HW, mg)	1282 \pm 37	1176 \pm 23*	1231 \pm 26	1065 \pm 48**
L.V.+S. weight (mg)	983 \pm 21	863 \pm 14**	902 \pm 20	778 \pm 37*
HW/BW (mg/g)	4.62 \pm 0.05	4.50 \pm 0.06	4.30 \pm 0.05	4.31 \pm 0.05
L.V.+S. weight/BW (mg/g)	3.54 \pm 0.04	3.30 \pm 0.04**	3.15 \pm 0.05	3.15 \pm 0.05

Values are the mean \pm S.E.M. * p <0.05; ** p <0.01, compared with each normal-diet group (unpaired t -test). L.V. left ventricle; S., Septum.

though the heart weight-to-body weight ratio was not changed by sesamin feeding, the L.V.+septum weight-to-body weight ratio was slightly but significantly decreased by sesamin feeding.

Effects of Dietary Sesamin on Body Weights and Heart Weights of Salt-Unloaded SHRSP In the salt-unloaded series, a gain in body weight of the sesamin-diet group was smaller than that in the normal-diet group. The heart weight and L.V.+septum weight in the sesamin-diet group was also significantly smaller than in the normal-diet group. However, the heart weight-to-body weight ratio and the L.V.+septum weight-to-body weight ratio were not changed by sesamin feeding (Table 1).

Effects of Dietary Sesamin on the Vascular Hypertrophy of Salt-Loaded SHRSP The data of morphometric analysis of the aorta and mesenteric artery in salt-loaded normal- and sesamin-diet groups are summarized in Table 2. Sesamin feeding slightly but significantly decreased the wall thickness and wall area of the aorta. Similar results were observed in the case of the mesenteric artery.

Effects of Dietary Sesamin on the Vascular Hypertrophy of Salt-Unloaded SHRSP Sesamin feeding in the salt-unloaded group slightly but significantly decreased the wall area of the aorta. The wall thickness of the aorta also tended to decrease in the sesamin-diet group, although the changes were not statistically significant. On the other hand, sesamin feeding produced no remarkable morphometric changes in

the mesenteric artery (Table 2).

Effects of Dietary Sesamin on the Histological Renal Damage of Salt-Loaded SHRSP Figure 3 shows typical examples of light micrographs of the renal tissues of salt-loaded normal- and sesamin-diet groups. In the normal-diet group, there were thickened tunica intima (Fig. 3a) and fibrinoid degeneration (as deeply stained) of the arterial wall (Fig. 3c) in small arteries. Figures 3b and 3d revealed normal arteries in renal tissues from sesamin-fed animals. There were no histological differences in the glomerulus and tubules between the two groups (data not shown). The data of histological renal damage are summarized in Table 3. The incidence of thickened tunica intima of small arteries was markedly decreased by the sesamin feeding. In addition, fibrinoid degeneration of the arterial wall was not observed in the renal tissues of sesamin-fed animals.

Effects of Dietary Sesamin on the Histological Renal Damage of Salt-Unloaded SHRSP When a histological study was performed on the kidneys of salt-unloaded SHRSP, medial thickening of the interlobular artery and fibrinoid necrosis of the arcuate artery (or interlobular artery) were often observed, although the severity of the renal damage was mild. Sesamin feeding slightly decreased the incidence of these histological changes but the effect was not statistically significant.

Table 2. Morphological Analysis of Aorta and Mesenteric Artery in Normal- and Sesamin-Diet Groups

Group	(n)	Aorta		Mesenteric artery	
		Wall thickness (μm)	Wall area (mm ²)	Wall thickness (μm)	Wall area (mm ²)
Salt-loaded (1% NaCl)					
Normal-diet	(7)	136±3	0.764±0.018	80.2±1.5	0.219±0.005
Sesamin-diet	(7)	124±3*	0.670±0.017**	73.6±1.6*	0.201±0.005*
Unloaded (H ₂ O)					
Normal-diet	(5)	132±3	0.678±0.023	80.9±0.9	0.177±0.006
Sesamin-diet	(6)	121±4	0.598±0.025*	79.0±2.0	0.169±0.008

Values are the mean \pm S.E.M. * $p < 0.05$; ** $p < 0.01$, compared with each normal-diet group (unpaired t -test).

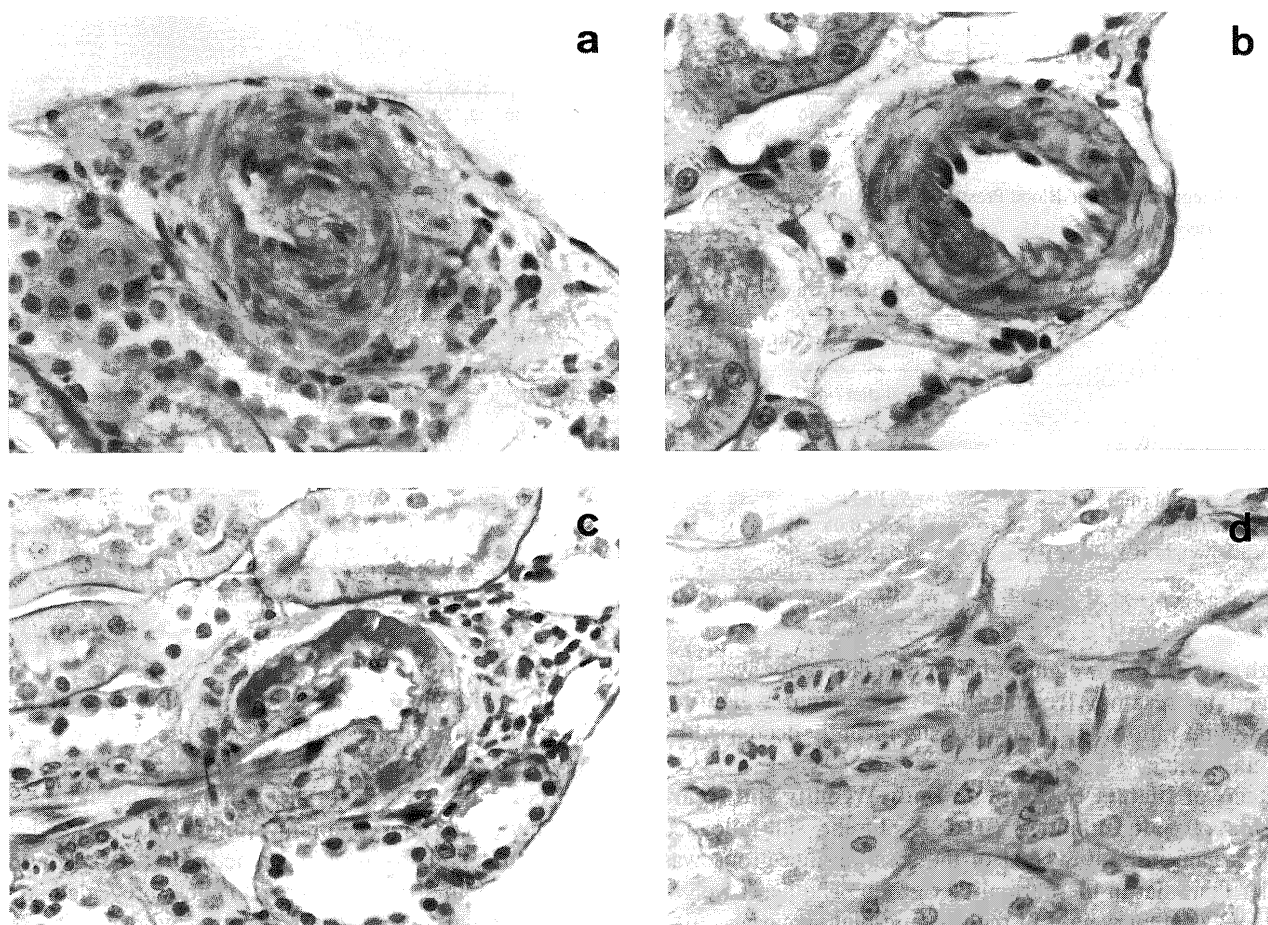


Fig. 3. Representative Light Micrographs of Renal Tissues Obtained from Salt-Loaded SHRSP Fed Normal-Diet or Sesamin-Diet

a, thickened tunica intima of small artery in normal-diet group ($\times 600$); b, d, normal small artery in sesamin-diet group ($\times 600$); c, fibrinoid degeneration of small arterial wall in normal-diet group ($\times 600$).

Table 3. Histological Renal Damage in Salt-Loaded Normal- and Sesamin-Diet Groups

Group Sample No.	Normal-diet							Sesamin-diet						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number of arteries with thickened tunica intima (mean \pm S.E.M.)	14	13	7	13	11	13	12	6	3	4	3	8	5	6
				(119 \pm 0.9)						(5.0 \pm 0.7*)				
Fibrinoid degeneration of arterial wall ^{a)} (incidence)	0	0	1	1	1	1	0	0	0	0	0	0	0	0
				(4/7)						(0/7 [#])				

* $p < 0.01$, compared with normal-diet group (Mann-Whitney-test). # $p < 0.01$, compared with normal-diet group (χ^2 -test). a) Grade of findings 0: none, 1: mild.

DISCUSSION

We have recently demonstrated that dietary sesamin efficiently suppresses the development of hypertension and cardiovascular hypertrophy induced by DOCA and salt.⁶⁾ In particular, the ameliorating effects of sesamin feeding on vascular hypertrophy (as determined by measuring wall thickness, wall area and the wall-to-lumen ratio of the aorta and mesenteric artery) were markedly effective in DOCA-salt hypertensive rats, in contrast to those seen in 2K, 1C renal hypertensive rats.⁷⁾ In the present study, we investigated the effect of sesamin feeding on the pathogenesis of both salt-loaded and unloaded SHRSP. The results clearly indicated that sesamin feeding to salt-loaded SHRSP markedly delayed the development of hypertension and ameliorated both vascular hypertrophy and renal damage, compared with salt-unloaded SHRSP. Taken together, it seems likely that sesamin efficiently delays the malignant status of hypertension and/or salt-dependent pathological condition.

In both salt-loaded and unloaded groups, sesamin feeding decreased heart weight. This alteration seems to be mainly due to the small gain of body weight in the sesamin group. This resulted in nonsignificant changes in the heart weight-to-body weight ratio. On the other hand, the L.V.+septum weight-to-body weight ratio was slightly but significantly decreased by sesamin in the salt-loaded group. Less potent decreasing effects of sesamin on the cardiac hypertrophy in SHRSP than in DOCA-salt⁶⁾ and 2K, 1C renal hypertensive rats⁷⁾ may be related to the antihypertensive activities.

The mechanisms by which sesamin exhibits antihypertensive activity are unclear. Several humoral factors such as the renin-angiotensin system are known to play an important role in the development of hypertension and cardiovascular organ damage.⁹⁾ However, it is unlikely that sesamin exerts antihypertensive action by interfering with the renin-angiotensin system, since this lignan is more effective on renin-independent DOCA-salt hypertension than on the renin-dependent 2K, 1C renal hypertensive model.^{6,7)} In our separate experiments using the rat aortic ring, sesamin produced Ca^{2+} -antagonistic vasorelaxing activity (unpublished data). This pharmacological action, at least in part, may contribute to its antihypertensive activity.

Several studies have suggested a relationship between hypertensive diseases and free oxygen radicals.^{10,11)} The increased production of a superoxide radical in vascular tissues leads to the attenuation of endothelium-dependent vasorelaxation, probably *via* the inactivation of endothelium-derived nitric oxide, and therefore, endothelium-dependent vasorelaxation is enhanced by superoxide dismutase.^{12,13)} More recently, it has been reported that the intravenous administration of superoxide dismutase with a high affinity for endothelial cells to spontaneously hypertensive rat (SHR) exerts an antihypertensive action,¹⁴⁾ thereby suggesting that superoxide radicals in vascular endothelial cells play a critical role in the

pathogenesis of the hypertension of SHR. It has been reported that the administration of α -tocopherol can suppress the development of hypertension in SHRSP.¹⁵⁾ Hirose *et al.*⁴⁾ noted the antioxidative effect of sesamin, the activity of which is comparable to that of α -tocopherol. Thus, the antioxidative effect of sesamin may contribute to its antihypertensive activity.

Age-related increases in blood pressure in salt-loaded SHRSP were not different from those of non-salt-loaded SHRSP. These results are consistent with a recent report from another laboratory.¹⁶⁾ On the other hand, salt-loading is one of the risk factors for tissue or organ damage.¹⁷⁾ We also noted the cardiovascular hypertrophy and renal vascular damage in salt-loaded SHRSP. In the present study, sesamin feeding efficiently prevented the development and maintenance of hypertension in salt-loaded SHRSP, although the blood pressure of the normal- and sesamin-diet groups was at almost the same level at 30 weeks. This antihypertensive effect during the sesamin-feeding period appears to contribute to the suppressive effects on concomitant cardiovascular hypertrophy and renal damage observed in salt-loaded SHRSP. Based on the salt-dependent pathological mechanisms being responsible for the essential hypertension, the possibility that sesamin is useful as a protective agent against hypertension in humans warrants further attention.

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