EFFECTS OF HIGH OR LOW SODIUM INTAKE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Spontaneously hypertensive rats (SHR) developed and maintained hypertension in spite of low sodium intake. The development of hypertension was accelerated and hypertension was enhanced by chronic excess sodium intake (1% salt in drinking water with or without high sodium diet). Various substrains of SHR showed some difference in sensitivity to salt ingestion. Consequently, hypertension in SHR is mainly determined by genetic factors, the minor part of which interacts with excess salt intake especially in several substrains of SHR.

THE strain of spontaneously hypertensive rats (hereafter abbreviated as SHR), separated by *Okamoto* and *Aoki*,¹ was started by the mating between a male Wistar rat from the Animal Center Laboratory, Kyoto University (hereafter abbreviated as Wistar-Kyoto or WK) with mild hypertension (145–175 mmHg) and a female with relatively high blood pressure (130–140 mmHg), and the most severe hypertensive rats in the subsequent litters were selected for sib-mating. One hundred per cent occurrence of hypertension with the subsequent development of hypertensive cardiovascular lesions in the third generation and thereafter showed that SHR had hypertension as a cardiovascular disease and they were studied from various aspects.² This strain was finally established as an inbred strain after 20 generations of sib-breeding³ and has now reached the F₂₈ generation. The establishment of the hypertensive strain itself indicated that the most important factors for the development of hypertension were genetic ones and the detailed

mode of the heredity of hypertension was studied in SHR.^{3–5}

Hypertension in animals is experimentally produced by many manipulations, such as in renal, renoprival, adrenal regeneration, DCA, neurogenic and salt hypertensions. These hypertensions are equivalent to secondary hypertension in human beings. On the other hand, SHR, in which genetic factors greatly contribute to the development of hypertension, seem to be the best model for the study on human essential hypertension^{1–3} and this was supported by hemodynamic studies of *Folkow et al.* on SHR.⁶ As in essential hypertension, possible interactions between genetic and environmental factors were supposed to be in SHR, although the degree of genetic determination of blood pressure was as high as 80–90%.^{3–5} Extreme alterations of environmental factors such as various stress loadings⁷ and high fat-cholesterol-salt diet feedings^{8,9} were confirmed to affect the development of hypertension and/or hypertensive cardiovascular complications.

Especially, the relationship between salt and hypertension in man^{10–12} has attracted much attention. Experimental chronic salt loading pro-

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duced hypertension in animals,^{13,14} and the roles of genetic predisposition and of sodium intake for the development and maintenance of hypertension were investigated in hypertension-prone rats by *Dahl et al.*^{15,16} and also in SHR¹⁷⁻²⁰. As there seems to be a little discrepancy among the conclusions of the previous reports as to the sensitivity of SHR to salt ingestion, the present studies were undertaken to reevaluate precisely the role of sodium intake and its interaction of genetic factors in the development of hypertension by observing 1) effect of low and high sodium diet on the development of hypertension, and 2) effect of salt loading on blood pressure in the various substrains of SHR with different genetic traits.^{3,8,9,21,22}

MATERIALS AND METHODS

Male spontaneously hypertensive rats (*Okamoto* and *Aoki*) (SHR)¹⁻³ were used with normotensive rats of Wistar-Kyoto, from the ancestors of which SHR had been separated. In the first experiment 16 male SHR (F₄) were divided into 4 groups which were fed on the following diets from the 5th week of age: group 1; low sodium diet (0.079%), group 2; standard sodium diet (0.276%), groups 3 and 4; high sodium diet (2.76%). All diets were supplied from the Orien-

tal Yeast Co., Tokyo. Groups 1, 2 and 3 were given tap water for drinking and libitum and group 4 was given 1% salt in drinking water. In the second experiment 17 to 31 male SHR (F_{25,26}) of A₁, A₁-sb, A₂, A₃, B₁, B₂, B₂-ob, and C substrains^{3,8,9,21,22} respectively, were used with 14 male rats of Wistar-Kyoto (WK). SHR from each substrain and WK were divided into two groups at the age of 6 to 8 weeks and one group was loaded with 1% salt in the drinking water thereafter for 15 weeks, while the other was given tap water. They were fed on standard stock chow diet CA-1 (Japan CLEA Co., Tokyo).

All rats were housed under stable conditions of temperature (22-25°C) and humidity (50-60%). Blood pressure was measured indirectly by the tail-plethysmographic method¹ once weekly without anesthesia.

RESULTS

1. Effect of high or low sodium diet on the development of spontaneous hypertension (Fig.1)

Although no difference in blood pressure was noted among groups 1 (low sodium diet), 2 (standard sodium diet), 3 (high sodium diet) and 4 (high sodium diet + 1% salt in drinking water)

Fig.1. Effects of sodium intake on blood pressure in male spontaneously hypertensive rats fed on diet containing low sodium (0.079%), standard sodium (0.276%) and high sodium (2.76%) with or without 1% salt in drinking water.

at the beginning of this experiment, group 4 developed hypertension earlier than the others as shown in Fig.1. At the 5th week of experiment (10 weeks after birth), blood pressure in group 4 (204 ± 8 mmHg, $M \pm SD$) was significantly ($p < 0.01$) higher than those in groups 1 (170 ± 13), 2 (172 ± 16) and 3 (173 ± 11). During 30 weeks of experiment there was no significant difference in blood pressure in groups 1, 2 and 3. SHR developed hypertension without any excess salt intake (group 2) and even under dietary condition of low sodium (group 1). Neither effective acceleration of the development of hypertension nor augmentation of its grade was observed in

group 3 fed on high sodium diet (2.76%). Sodium effect on hypertension was more marked when the drinking water was additively loaded with 1% salt than when diet only was loaded but not the drinking water.

Growth was slightly slowed down in the low and high sodium diet groups compared with that in the standard diet group 2. Their body weights in gram at the 5th (and 20th) weeks of experiment were as follows ($M \pm SD$): group 1; 215 ± 7 (316 ± 16), group 2; 303 ± 20 (363 ± 10), group 3; 263 ± 10 (305 ± 10) and group 4; 214 ± 35 (267 ± 32).

All rats were alive at the 15th week of experi-

TABLE I BLOOD PRESSURE IN MALE SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND MALE WISTAR-KYOTO RATS (WK) LOADED WITH OR WITHOUT 1% SALT IN DRINKING WATER

Strain or substrains	Age at the start of experiment (weeks)	Salt loading	Duration of salt loading (weeks)			
			0	5	10	15
SHR	A_1	+	187 ± 13 (17)	198 ± 11 (17)	217 ± 19 (9)*	225 ± 25 (7)*
		-	182 ± 7 (14)	199 ± 9 (14)	200 ± 11 (10)	199 ± 13 (9)
	A_{1-sb}	+	178 ± 6 (10)	213 ± 8 (9)**	248 ± 16 (4)**	255 ± 14 (3)**
		-	184 ± 9 (12)	199 ± 10 (10)	201 ± 7 (11)	200 ± 10 (7)
	A_2	+	191 ± 11 (12)	209 ± 20 (11)*	222 ± 31 (3)	222 ± 12 (3)*
		-	180 ± 9 (5)	188 ± 9 (5)	199 ± 8 (5)	207 ± 6 (5)
	A_3	+	189 ± 10 (13)	199 ± 17 (12)	232 ± 28 (6)*	240 ± 10 (3)**
		-	182 ± 8 (7)	194 ± 12 (7)	206 ± 13 (7)	208 ± 5 (7)
	B_1	+	168 ± 5 (13)	226 ± 24 (12)**	237 ± 25 (25)**	228 ± 15 (3)**
		-	170 ± 4 (12)	189 ± 9 (7)	197 ± 7 (12)	204 ± 6 (12)
	B_2	+	179 ± 7 (19)	200 ± 14 (20)	213 ± 32 (13)	220 ± 21 (6)*
		-	184 ± 13 (7)	191 ± 13 (7)	198 ± 8 (5)	200 ± 9 (5)
WK	B_{2-ob}	+	177 ± 6 (12)	203 ± 9 (12)	241 ± 10 (6)**	248 ± 16 (16)**
		-	177 ± 7 (10)	196 ± 13 (9)	193 ± 8 (9)	204 ± 7 (5)
	C	+	180 ± 5 (10)	201 ± 10 (10)**	231 ± 16 (7)**	254 ± 15 (7)**
		-	177 ± 5 (15)	184 ± 10 (12)	196 ± 11 (15)	204 ± 8 (11)
	7	+	118 ± 6 (9)	122 ± 10 (9)	132 ± 4 (9)**	131 ± 4 (6)**
		-	118 ± 6 (5)	118 ± 5 (5)	121 ± 5 (5)	122 ± 4 (5)

$M \pm SD$

(): Number of rats checked for blood pressure.

**, *: Statistically significant differences ($P < 0.01$, $0.01 < P < 0.05$, respectively) from the non-salt-loaded group in the same substrain.

Fig.2. Blood pressure in male spontaneously hypertensive rats (SHR) and male normotensive Wistar-Kyoto rats (WK) loaded with or without 1% salt in drinking water for 15 weeks.

(): Number of rats checked for blood pressure.

Statistical differences: a (a'), b (b'), c (c'), d (d'), e (e'), f (f'), g (g'), h (h') and i (i') show statistically significant differences, $p < 0.01$ ($p < 0.05$), from the values of A₁, A₁-sb, A₂, A₃, B₁, B₂, B₂-ob, C and WK, respectively.

TABLE II RELATIONSHIP BETWEEN SALT SENSITIVITY AND TOXICITY IN VARIOUS SUBSTRAINS IN SPONTANEOUSLY HYPERTENSIVE RATS

Salt toxicity [Mortality rate]	Salt sensitivity (Blood pressure)	
	High (248–255)	Low (220–228)
High [62–83]	A ₁ -sb [70] (255)	A ₂ [83] (222) B ₁ [62] (228)
	A ₃ [43] (240)	
Low [0–33]	C [0] (254) B ₂ -ob [20] (248)	A ₁ [17] (225) B ₂ [33] (220)

(): Blood pressure in mmHg after 15 weeks of 1% salt loading in drinking water.

[]: Mortality rate in % during 15 weeks of the salt loading.

ment. However, the life span was shortened in the groups fed on low or high salt regimen and final mortality rates at the 45th week of experiment were as follows: group 1 (50%), 2 (25%), 3 and 4 (100%).

2. Effect of high salt intake on blood pressure in various substrains of SHR (Table I, II and Fig.1)

SHR (F_{25,26}) at present are classified into the following 8 substrains; A₁, A₁-sb, A₂, A₃, B₁, B₂, B₂-ob and C, according not only to genealogy, but also to isozyme analyses, responses to hypercholesterolemic diet, susceptibility to hypertensive cardiovascular lesions and others.^{3,8,9,21,22} Non-salt-loaded groups of each substrain developed almost a similar grade of hypertension. Although there were some statistically significant differences in blood pressure among these substrains at 0, 5th and 10th week of experiment, no significant difference was observed at the 15th week of experiment (21–23 weeks after birth) when gene actions were supposed to be fully developed in SHR.⁴

One per cent salt loading in drinking water accelerated the development of hypertension and enhanced hypertension in all substrains of SHR and also in normotensive rats of Wistar-Kyoto as shown in Table I. Salt-loaded groups of A₁-sb, A₂, B₁ and C showed significantly higher blood pressure than the non-salt-loaded group of each substrain even at the 5th week of experiment. In all salt-loaded groups including WK, increases in blood pressure were significantly greater than those in non-salt-loaded control groups at the 15th week of experiment. As blood pressures in non-salt-loaded groups were not different among substrains at this time, blood pressures in salt-loaded groups were compared with each other among substrains (Fig.2). The groups with average blood pressure over 240 mmHg were A₁-sb (255 mmHg), C (254 mmHg), B₂-ob (248 mmHg) and A₃ (240 mmHg), while those with average blood pressure below 230 mmHg were B₁ (228 mmHg), A₁ (225 mmHg), A₂ (222 mmHg) and B₂ (220 mmHg). As some of the former groups, A₁-sb, C and B₂-ob showed a significant difference in blood pressure from some of the latter groups, A₂ and B₂, the former substrains were considered to be rather more sensitive to salt loading than the latter substrains.

As for salt toxicity many rats died of cardiovascular lesions 5–10 weeks after the start of salt loading. Mortality rates were calculated at

the 15th week of experiment and is shown in Table II in relation to the above-mentioned relative salt sensitivity. All cases were checked by autopsy and the detailed findings of histopathological examination on hypertensive cardiovascular lesions are to be reported elsewhere. Although a parallelism between salt toxicity and sensitivity was noted in A₁-sb with high toxicity and sensitivity, A₃ with intermediate toxicity and sensitivity and also in A₁ and B₂ with low toxicity and sensitivity, there seemed to be a discrepancy, too, between salt sensitivity and toxicity in C and B₂-ob with high sensitivity and low toxicity as well as in A₂ and B₁ with low sensitivity and high toxicity.

DISCUSSION

Although no definite conclusion has been obtained yet on the role of salt in the pathogenesis of essential hypertension in human beings, genetic factors are evidently important in essential hypertension^{23,24} and also in spontaneous hypertension in animals.^{1–5,25–28} In SHR, hypertension is inherited additively and mainly determined by a relatively small number of major genes.^{3–5} The present study showed that hypertension developed without the aid of excess salt ingestion and even under sodium restriction, supported the previous reports^{17,19,20} and confirmed the importance of genetic factors in hypertension of SHR. In this point SHR is different from Dahl's sensitive strain in which an interaction between genes and environmental factors (chronic salt ingestion) is indispensable for the development of hypertension.^{15,16}

In regard to the sensitivity to chronic salt ingestion in SHR, there has been a little difference in the conclusions of the previous reports on SHR whether they are sensitive^{18,19} or rather insensitive.¹⁷ This debate seems mainly to come from the difference in experimental regimens of sodium loading. The present experiment showed that high sodium diet (2.76%) plus plain water for drinking was ineffective, while the same high sodium diet plus 1% salt in drinking water or only the latter was evidently effective for the augmentation of hypertension. The previous experiment using high salt diet without salt in drinking water¹⁷ resulted in the insensitivity to salt ingestion and the experiments using 1% salt in drinking water^{18,19} confirmed the sensitivity in SHR. One per cent salt in drinking water is more effective than 1% sodium diet plus plain water. It may be simply because sodium in diet

is diluted for excretion with an increased amount of water taken together, for exaggerated natriuresis follows sodium loading both in hypertensive men²⁹ and in SHR.³⁰

The augmentation of hypertension by chronic sodium ingestion was observed in SHR and an interaction between hypertensinogenic genes and loaded sodium was presumed to exist also in SHR. As sensitivity to excess salt intake was different among various substrains of SHR, genetic factors involved in sodium metabolism might be somewhat different among these substrains. These substrains have some difference in susceptibility to hypertensive vascular lesions; for example, A₁-sb and A₃ are vulnerable while B₂ including B₂-ob is rather resistant to the lesions^{3, 8, 9, 21, 22}, although their blood pressure levels are similar to each other. Chronic salt ingestion is toxic, for it induces cardiovascular lesions,³¹ especially in hypertension.³² Salt toxicity and sensitivity were observed in this experiment as mortality rate and enhancement of hypertension, respectively. These substrains can be tentatively classified into 4 groups, with high sensitivity and toxicity, with low sensitivity and toxicity, with high sensitivity and low toxicity, and with low sensitivity and high toxicity. It is noteworthy that there is a discrepancy between salt sensitivity and toxicity in some substrains such as A₂ and B₁ with high toxicity and low sensitivity, as well as C and B₂-ob with high sensitivity and low toxicity. The former might have expired before they showed enhanced hypertension because of salt toxicity. However, the latter substrains seemed to be resistant to salt-induced vascular lesions in spite of the augmentation of hypertension. Therefore, genetic disposition about salt toxicity and sensitivity might be independent from each other. Further separation of the substrains with these extreme characteristics, and the detailed analysis of substrain-difference in salt toxicity and sensitivity are expected to contribute to the clarification of an interaction between genetic disposition and excess salt intake for the induction of hypertensive vascular diseases such as cerebral hemorrhage and softening.

SUMMARY

Effects of sodium restriction or chronic sodium excess on blood pressure were observed in male spontaneously hypertensive rats (Okamoto and Aoki) (SHR) and various substrains of SHR in order to clarify the role of sodium and its interaction with genetic factors in spontaneous hyperten-

sion, and the following results were obtained.

- 1) Hypertension developed in SHR fed on low sodium diet (0.079%) and the grade of hypertension in these rats was not greatly different from that in SHR fed on standard or high sodium diet (0.276 or 2.76%, respectively) with tap water for drinking.
- 2) Development of hypertension was accelerated and its grade was augmented in SHR fed on high sodium diet (2.76%) with 1% salt in drinking water.
- 3) Hypertension in various substrains of SHR was enhanced by 1% salt loading in drinking water for 5 to 15 weeks.
- 4) Some substrains of SHR were more sensitive to the same salt regimen than the others, and a discrepancy between salt sensitivity and toxicity was observed in some substrains of SHR.
- 5) These results indicated that hypertension in SHR was genetically determined to the major extent and that alterations of salt intake interacted with genetic predisposition to modify the grade of hypertension or the course of hypertensive vascular diseases.

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