Blood Pressure and Sympathetic Nervous Function in Spontaneously Hypertensive Rats Derived from Breeders on Low Sodium Diet

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We investigated the effect of dietary salt restriction in spontaneously hypertensive rat (SHR) breeders to determine if the development of hypertension of offspring would be blunted. Weaning SHRds (F-0) were divided into 3 groups and were given a diets containing sodium of 0.4% (G 1), 0.05% (G 2), or 0.4% plus meprosate 0.001% (G 3) with distilled water. Potassium content was 0.75% and the other ingredients except chloride were identical in all the diets. Offsprings (F-1) were derived from selective inbreeding within each group. Systolic blood pressure rose over 160 mmHg by 8 weeks of age in all the male rats of all the groups in both F-0 and F-1. There was no difference in blood pressure between G 1 and G 2 at any age, while in G 3 blood pressure was significantly lower than in G 1 at 12 and 20 weeks of age in F-1. The findings in pressure were ascertained by heart-body weight ratio determined at autopsy, which was similar between G 1 and G 2, and was smaller in G 3 than the others at 20 weeks of age. Aldosterone excretion rate was markedly higher in G 2 than G 1 and G 3 at all study points, whereas plasma renin content was similar between G 1 and G 2, and higher in G 3. Plasma concentrations of noradrenaline and adrenaline did not differ among the 3 groups at any age in both F-0 and F-1. There was also no difference in cardiovascular responsiveness to intravenous noradrenaline or hexamethonium among the groups at any age in both F-0 and F-1. It is concluded that life-long sodium restriction does not blunt the development of hypertension in SHR, which might be attributed to failure of suppression in sympathetic nervous function.

HIGH sodium diet exacerbates hypertension in spontaneously hypertensive rats (SHR, Okamoto-Aoki strain); however, sodium deprivation in the diet does not ameliorate hypertension, unless the dietary sodium is so low to affect overall growth.¹ The purpose of the present investigation was to determine whether or not 1) dietary sodium deprivation in the breeders, from weaning to adulthood, affects the development of hypertension in the offspring in SHR, 2) plasma concentrations of renin and catecholamines in crease in those rats 3) cardiovascular responsiveness to exogenous noradrenaline and hexamethonium are changed in those rats.

Male and female SHR/Sea at weaning (F-0) were obtained from Seiwa Experimental Animals

Key Words:
SHR
Breeding on low Na
Renin
Catecholamines
Vascular responsiveness

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Ltd., Japan. They were randomly assigned to 3 groups and fed special diet (Oriental Kobo Co. Ltd.) containing sodium of 0.4% (G 1), 0.05% (G 2), or 0.4% plus mefruside (a diuretic) of 0.001% (G 3) and given distilled water to drink ad libitum. Potassium content was 0.75% and other dietary ingredients except chloride were identical for all diets. Offsprings (F-1) were derived from selective inbreeding within each group. Only male rats were used for the study.

Does Dietary Sodium Restriction Prevent Hypertension in SHR?

Total numbers of the male rats used for G 1, G 2, G 3 were 15, 16 16 in F-0, and 16, 16, 16 in F-1, respectively. Food and water consumption, which was determined by housing each rat separately in a single metabolic cage for 24 hours at 6, 8, 12 and 20 weeks of age, did not differ among the groups. An average of mefruside taken by the rats in G 3 (mg/kg body weight/24 hours) at 6, 8, 12, 20 weeks of age was 26.9, 17.1, 14.0, 10.2 in F-0 and 24.4, 19.9, 12.1, 11.0 in F-1, respectively.

Body weight was similar in G 2 compared to G 1 up to 20 weeks of age in both F-0 and F-1, though it tended to be larger in G 2 than G 1 up to 12 weeks of age in F-1 (not significant). It was smaller in G 3 than in G 1 at 12 weeks of age and thereafter in F-0 (p < 0.05–0.01), and also at 12 and 20 weeks of age in F-1 (p < 0.01). An average of body weight (g) at 20 weeks of age for G 1, G 2, G 3 was 310.6 ± 2.7, 316.9 ± 4.0, 290.2 ± 2.2 in F-0, and 291.4 ± 3.7, 298.9 ± 3.5, 270.9 ± 2.7 (Mean ± SEM) in F-1, respectively.

Systolic blood pressure (tail-cuff method) rose to over 170 mmHg in all groups by 8 weeks of age in F-0. There was no significant difference in blood pressure between groups up to 20 weeks, though blood pressure tended to be lower in G 3. Systolic blood pressure (mmHg) at 20 weeks of age for G 1, G 2, G 3 was 184.0 ± 2.9, 189.1 ± 2.7, 179.0 ± 1.4 (Mean ± SEM), respectively. In F-1, systolic blood pressure rose over 160 mmHg in all groups by 8 weeks of age. Blood pressure in G 2 was higher than in G 1 at 8 weeks of age (p < 0.05), followed by a similar increase in blood pressure as in G 1 thereafter. It was significantly lower in G 3 compared to G 1 at 12 and 20 weeks of age (p < 0.01). Systolic blood pressure at 20 weeks of age for G 1, G 2, G 3 was 197.4 ± 2.7, 199.9 ± 2.9, 176.8 ± 2.4, respectively.

Heart rate decreased concomitantly with blood pressure rising at 8 weeks of age in all groups of F-0 and F-1, and was restored gradually to the initial level at 6 weeks of age. There was no significant difference in heart rate among the groups at all ages except at 12 weeks of age in F-1 when heart rate was greater in G 3 than G 1 (p < 0.01).

The 24 hour excretion of urinary sodium was markedly less in G 2 than G 1 (p < 0.01) and was similar in G 3 compared to G 1 throughout the observation period in both F-0 and F-1, while potassium excretion was similar among the 3 groups at all ages. Urinary sodium-potassium ratio (Na/K/24 hours) was lower in G 2 than in G 1 at all the points examined (p < 0.01), while in G 3 it was similar to G 1 until 12 weeks of age in F-0 and until 8 weeks of age in F-1 when it increased.

Urinary creatinine excretion was less in G 3, however, creatinine coefficient (mg/100g body weight/24 hours) showed no difference among the groups. Urinary sodium per creatinine (μEq/mg creatinine/24 hours) was consistently lower in G 2 than in G 1 (p < 0.01), and was higher in G 3 (p < 0.05–0.01).

Aldosterone excretion rate (AER) was always higher in G 2 than in G 1 both in F-0 and F-1 (p < 0.01). AER in G 3 was higher than in G 1, and lower than that in G 2 in both F-0 and F-1 (p < 0.05–0.01). AER (ng/24-hours) for G 1, G 2, G 3 in F-1, for example, was 21.3 ± 1.05, 49.2 ± 3.06, 28.9 ± 1.34 at 8 weeks of age, and 31.0 ± 1.28, 64.3 ± 4.39, 36.5 ± 2.49 at 20 weeks of age, respectively.

Our results demonstrate that a sodium restriction over one generation failed to prevent hypertension in SHR, and also that treatment with a diuretic produced some attenuation of blood pressure. Aldosterone is very important to maintain sodium homeostasis in chronically sodium-restricted state.

Heart Weight

Another set of SHR, every twelfth rat from each group in F-1, was sacrificed at 8, 12 and 20 weeks of age, and heart was weighed. Neither heart weight nor heart-body weight ratio was different between G 1 and G 2 at any age. Heart body weight ratio as well as heart weight was lower in G 3 than G 1 at 12, 20 weeks of age (p < 0.01). Heart-body weight ratio (mg/100g) for G 1, G 2, G 3 at 20 weeks of age was 350 ± 3.6, 350 ± 4.0, 320 ± 3.5, respectively.

Thus, the similarity in blood pressure elevation and body growth between G 1 and G 2 SHR, and also an attenuation of blood pressure in G 3 SHR
were ascertained by these data on heart weight.

**Plasma Concentration of Renin and Catecholamines**

Arterial blood was drawn in conscious state through the indwelling catheter in the right carotid artery of the rat for determinations of plasma renin content (PRC) and plasma concentration of noradrenaline (NA) and adrenaline (Ad). PRC was determined by radioimmunoassay and catecholamines by radioenzymatic assay. The examinations were made in every twelfth rat of G 1, G 2, G 3 at 8, 12, 20 weeks of age in F-0 and F-1.

PRC in G 2 was similar to that in G 1 at all ages in both F-0 and F-1 except that at 8 weeks of age in F-0, PRC was higher in G 2 than G 1 (33.2 ± 3.7 vs 13.9 ± 1.5 ng/ml/hour, p < 0.05) and not different between G 2 and G 3 (38.9 ± 5.1). PRC in G 3 was consistently higher than that of G 1 in both F-0 and F-1. PRC for G 1, G 2, G 3 at 20 weeks of age was 14.5 ± 1.8, 21.2 ± 1.5, 96.3 ± 12.6 in F-0, and 14.7 ± 1.6, 15.0 ± 0.9, 72.8 ± 5.9 in F-1, respectively.

Neither NA nor Ad was significantly different among these 3 groups at any age in F-0 and F-1. NA (pg/ml) for G 1, G 2, G 3 at 20 weeks of age was 844 ± 34, 895 ± 91, 864 ± 60 in F-0, and 610 ± 76, 572 ± 82, 538 ± 72 in F-1, respectively.

It is considered that chronic and life-long sodium restriction does not enhance PRC; higher PRC in G 2 of F-0 at 8 weeks of age reflected the acute effect of sodium restriction. This is in contrast with the results in G 3 rats treated with a diuretic, in which PRC was always higher than the other groups. The present results are consistent with our previous observations that PRC, as well as the effect of intravenous saralasin on arterial pressure, was not enhanced in normotensive Wistar rats bred successively on the same sodium restricted diet as in the present investigation. It has been suggested that long-term dietary sodium restriction may suppress sympathetic function; however, plasma catecholamines in the present study were not suppressed by sodium restriction and those levels were extremely high when compared to those of normotensive Wistar rats and were not suppressed by sodium restriction, indicating an important role of sympathetic nervous function to initiate hypertension in SHR.

**Responsiveness to Noradrenaline and Hexamethonium**

Cardiovascular responses to 1-noradrenaline and hexamethonium were examined in every tenth rat of G 1, G 2 and G 3 at 8, 12, 20 weeks of age in F-0 and F-1. Two catheters were inserted into a femoral artery and vein of the rat under ether anesthesia. At least 4 hours after recovery from anesthesia, mean arterial pressure (MAP) and heart rate (HR) were recorded by coupling the arterial line to a pressure transducer in conscious and unrestrained state. Intravenous injection was made through the venous catheter. The doses used were 0.3, 1.0, 3.0 µg/kg for 1-noradrenaline and 3 mg/kg for hexamethonium bromide, and the volume of administration was 0.1 ml for all drugs. Dose-response curve was obtained from each group by the log of the dose vs. the percent maximum change in MAP.

Intravenous injections of noradrenaline caused increases in MAP and reflex bradycardia to similar degrees in the 3 groups of SHR at all ages in both F-0 and F-1. The dose-response curves obtained from the 3 groups were overlapped at all ages and the X-intercept (log-dose at zero response) tended to rise following growth rates. The percent maximum decrease in MAP produced by hexamethonium injection at 20 weeks of age was 26.5 ± 2.3 in G 1, 25.1 ± 3.5 in G 2, 29.9 ± 2.8 in G 3 in F-0, and 23.7 ± 2.4, 23.4 ± 1.9, 25.4 ± 2.1 in F-1, respectively, and showed no difference among these groups. Hexamethonium effect at 8 and 12 weeks of age was also similar to each other among the groups in both F-0 and F-1.

Similarities in cardiovascular responses to intravenous noradrenaline or hexamethonium among the groups indicate that a sodium restriction over one generation as well as sodium deprivation by a diuretic does not cause a change in cardiovascular responsiveness to sympathetic stimuli in SHR. This contradicts the results of experiments on short-term sodium restriction.

In summary, dietary sodium restriction did not blunt the development of hypertension in SHR and caused no suppression in sympathetic nervous function. Sodium intake may have little influence on mechanisms of blood pressure regulation in SHR.

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