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Salt Water Promotes Hypertension in Dahl-S Rats

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Abstract: Hypertension was induced in Dahl-salt-sensitive (Dahl-S) rats by administering salt in drinking water. Control rats receiving tap water did not show a significant change in blood pressure or abnormalities in the kidney. Rats receiving 0.5% NaCl solution developed moderate hypertension and renal lesions. Rats receiving 1.0% NaCl solution showed prominent and increasing hypertension and severe renal damage. This method of salt administration should be simpler than administration in the diet as a means of promoting renal hypertension. The lower concentration salt water caused chronic mild hypertension in Dahl-S rats, and may serve as a useful model for progressive hypertension.

Key words: Dahl-salt-sensitive (Dahl-S) rats, renal hypertension, salt (NaCl) water

Dahl salt-sensitive (Dahl-S) rats, which develop hypertension due to the intake of salt (NaCl) in the diet [1, 2, 9], have come into wide use as an experimental model of renal hypertension. The progression of hypertension in these rats has generally been explained to result from a decrease in Na excretion by the kidneys [3, 4, 9].

To induce renal hypertension in Dahl-S rats, prescription diets containing NaCl are available. Another method of administering NaCl to Dahl-S rats is to supplement the drinking water with NaCl. In the diet-administered method, it is difficult to compound diets with various NaCl concentrations and costly to prepare the prescription diets. Dahl-S rats show no extreme decrease in water consumption despite their loss of appetite according to the progression of hypertension or renal failure [3, 4]. If NaCl is put into the water, more reliable NaCl administration can thus be obtained even

at the end stage of renal failure.

The purpose of this study was to induce renal hypertension in Dahl-S rats through providing NaCl containing drinking water and to regulate the degree of the hypertension by providing water with different NaCl concentrations.

This study was carried out in accordance with the Guide for Animal Experimentation, Faculty of Medicine, Kagoshima University.

Fifteen male Dahl-S/Sea rats were purchased at 4 weeks of age from a commercial breeder (Seiwa Experimental Animal, Japan). They were housed three per cage in wire-topped polycarbonate cages (350 × 400 × 180 mm) containing autoclaved wood shavings. The experimental room was an air-conditioned (temperature: 22 ± 2°C, humidity: 55 ± 10%, ventilation: 7 times/hr) room which was exposed to a 12 hr light/dark cycle.

At 5 weeks of age, the rats were divided randomly

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into three groups, group A ($n=6$) was given tap water, as the non-NaCl-administered control. Groups B ($n=3$) and C ($n=6$) were given 0.5% and 1.0% NaCl solutions instead of drinking water, respectively. The NaCl concentrations used were chosen after considering preliminary data about the tastes of the rats. All rats were given autoclaved commercial diet (CE-2; CLEA, Japan Inc.) and drinking water *ad libitum* throughout the experiment.

Diet and water intake, body weight, heart rate (HR) and blood pressure (BP) were measured once a week. Diet and water intake in each cage was measured and expressed in g/day/head as the average for each group. The BP and HR were measured by the tail cuff method (Ueda Inc.), in stationary rats (HR was 450 bpm) without anesthesia. Body weight and BP values were expressed as the mean $\pm$ SD, and differences among the three groups were analyzed statistically by factorial ANOVA. Differences with p -values less than 0.05 were considered to be statistically significant.

At 20 weeks of age, all rats were necropsied under sodium pentobarbital anesthesia. The heart and kidneys were carefully removed and weighed, the weights were measured by electronic balance (ER-120A, A&D company, Japan), fixed in 10% buffered formalin, and embedded in paraffin blocks. Slices of the specimens were stained with hematoxylin-eosin (H.E) and examined microscopically.

The diet intake were varied, at around 20 g/day/head in all groups, throughout this study. This was similar to previous studies [3–5]. The body weights increased consistently with the growth curve of Dahl-S rats fed ordinary diets or diets containing 4% NaCl.

The water intake is shown in Fig. 1. The intake in group A did not vary during this study, which was consistent with the results previously obtained in rats fed ordinary diets. The intake in group B was increased to between 50 and 65 g/day/head within 1 week. The intake in group C was somewhat higher (in the range 62–73 g/day/head) than the intake in groups A and B by 18 weeks of age, and thereafter increased further. In previous experiments, when the Dahl-S rats were placed on NaCl-containing diets, they exhibited polyposia, and the rats on higher NaCl-content diets drank more water [9]. The water intake in group C showed no decrease, which was consistent with previous data for rats fed 4% NaCl diets. The progression

Fig. 1. Water Intake (/day/head). Group A: tap water, Group B: 0.5% NaCl solution, and Group C: 1.0% NaCl solution.

Fig. 2. NaCl Intake (/day/head). Group B: 0.5% NaCl solution, Group C: 1.0% NaCl solution.

of hypertension in Dahl-S rat was more affected by the NaCl intake than the water intake. In this study, we estimated the NaCl intake in groups B and C (Fig. 2). In groups B and C, 250–325 mg/day/head and 620–730 mg/day/head NaCl, respectively, were taken daily. The NaCl intake in Group C was comparable to the intake in Dahl-S rats eating 20 g/day/head of 4% NaCl diet, i.e., about 800 mg/day/head [3]. These results suggested that the same salt loading can result from drinking 1.0% salt water as from eating a 4% NaCl diet.

The BP is shown in Fig. 3. In group A, the BP was not further increased after the rats reached 10 weeks of age. In groups B and C, the BP reached 187 ± 7.6 and 235 ± 26.4 mmHg, respectively, at 20 weeks of age. The increase in BP in group B was greater than that in

Fig. 3. Blood Pressure. Group A: tap water, Group B: 0.5% NaCl solution, and Group C: 1.0% NaCl solution.

Table 1. Heart and Kidney Weights (g)

	Group A	Group B	Group C
Heart	1.33 ± 0.15	1.64 ± 0.18	1.77 ± 0.10*
Left Kidney	1.55 ± 0.25	2.11 ± 0.31*	2.29 ± 0.15*
Right Kidney	1.54 ± 0.28	2.02 ± 0.20*	2.24 ± 0.19*

Group A: tap water, Group B: 0.5% NaCl solution, and Group C: 1.0% NaCl solution. *P<0.05 vs Group A.

group A, and the differences between groups A and B were significant at 16 and 18 weeks of age. Group C rats maintained a significantly higher BP than rats in groups A and B after the rats reached 10 weeks of age. In Hamada's study [3], rats fed 4% NaCl diets attained a BP of around 200 mmHg at 10 weeks of age, which was about the same (192 ± 4.0 mmHg) as that in the 1.0% salt water group in this study.

The heart and kidney weights are shown in Table 1. Group C rats had significantly heavier hearts than group A rats. The kidneys weighed significantly more in groups B and C than in group A. There were no abnormalities in the renal surface in groups A and B, but the renal surface in group C was rough, not glossy, and was yellowish-brown. Histopathological analysis of the renal tissues is shown in Photos 1 to 3. Group A had

Photo. 1

Photo. 2

Photo. 3

Photo. 1. Kidney in Group A. H·E staining. $\times 25$.

Photo. 2. Kidney in Group B. Mild tubular dilation and mild interlobular arterial hypertrophy were observed. H·E staining. $\times 25$.

Photo. 3. Kidney in Group C. Tubular dilation, hyaline casta, glomerulosclerosis and interlobular arterial hypertrophy were observed. H·E staining. $\times 25$.

no abnormalities in the kidneys (Photo 1). Group B had some renal lesions, which were milder and more restricted than those in group C (Photo 2). The renal lesions observed in group B were similar to those found in previous studies with diet-administered salt [6]. Group C demonstrated severe renal damage, including a thinner cortex, tubular dilation, proteinaceous tubular casts, glomerulosclerosis, interlobular arterial hypertrophy, and infiltration of neutrophils and lymphocytes (Photo 3). The findings in group C were suggestive of glomerular and tubular insults or arteriosclerosis, reported previously in Dahl-S rats which were fed 4% NaCl diets for 10 weeks [3]. These results indicated that 0.5% NaCl in the drinking water caused mild chronic hypertension in Dahl-S rats. Sustarsic also reported the development of mild hypertension in Dahl-S rats fed normal rat food containing 1% NaCl [11]. Our results also indicated that 1.0% NaCl in drinking water could promote renal hypertension as severe as that caused by eating a 4% NaCl diet.

In conclusion, the salt water method of administration may be a simpler way to regulate the degree of renal hypertension in Dahl-S rats and more economical than feeding prescription diets to the rats. Recent studies have demonstrated that prehypertensive Dahl-S rat

showed signs of other diseases, including hyperlipidemia and glomerulosclerosis [7, 8, 10]. In this study the lower concentration of salt water caused chronic mild hypertension in Dahl-S rats. This relatively slowly progressing hypertension model may be useful for studying other diseases prior to the development of more serious hypertension.

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