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INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF STROKE IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS (SHRSP): EFFECTS OF RESIDENTIAL CONDITION AND POPULATION DENSITY

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Stroke-prone spontaneously hypertensive rats (SHRSP) were bred in either wire-mesh cages (WMC) or plastic cages with wood-chips bedding (PWB), individually or in a group of 3 rats. The present study examined the effects of different residence conditions and population density on the development of hypertension, incidence of stroke, and on survival rate in the SHRSP given 1% NaCl water for drinking. As a result, the stroke or hypertensive encephalopathy was observed in all of rats. Life spans of the individual-housed and group-housed in WMC were 39 and 51 days, respectively, and those of the individual-housed and group-housed in PWB were 82 and 108 days, respectively. The PWB residence condition clearly delayed the development of severe hypertension and the incidence of stroke. No difference was found in body weight gain or blood pressure among the rats housed in either condition. These results demonstrated that the residential condition and population density affected the time to incidence of stroke and survival rate in SHRSP. Thus, it is suggested that the environmental condition related to emotional or psychosocial stress may cause biased experimental results.

Key words: environmental factors, housing, rats, SHRSP

INTRODUCTION

Interactions between genetic and environmental factors have been considered important for the pathogenesis of hypertension and stroke in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) (1, 2).

It has been well documented that environmental factors, especially nutritional factors, are important in the development of hypertension and cardiovascular diseases in the SHRSP. And these symptoms are prevented by the improvement of dietary conditions, such as high protein (3) and high-fat-cholesterol diet (4). Environmental factors, such as room temperature, relative humidity, wind velocity, ventilation, illuminance, and odor, are generally controlled in the animal institution (5). The residence materials (cage and bedding material) are one of the important environmental factors, because animals directly touch and may eat bedding materials. It is possible that the difference in residential condition has influences not only on the animal health and welfare, but also on the bias in experimental results. However, few studies have been reported regarding the effects of residential condition on hypertension and incidence of stroke in the SHRSP.

In the present study, we investigated the effects of differences in residence materials, stainless steel wire-mesh cage (WMC) vs. solid-bottomed plastic cage with wood-chip bedding (PWB), and differences in population density on the development of hypertension and stroke in the SHRSP.

MATERIALS AND METHODS

Animals

A total of 24 male SHRSP/N Crj, aged 10 weeks, were used. The animals have been the colony kept at the Institute of Experimental Animal of Shimane Medical University after they were originally obtained from Charles River Japan Inc. (Kanagawa, Japan). They were bred individually in PWB. Young

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animals were weaned from their mothers at 3-week-old and put in WMCs. They were kept in an animal room controlled at a constant condition (temperature: 23 ± 2℃, relative humidity: 50 ± 10%, and lighting times: 12 hr/day).

Residence materials

The experiment was carried out with two kinds of residence materials: one was WMC (260 W×370 D×170 H ㎜, CLEA Japan Inc., Tokyo), and the other was PWB (260 W×330 D×170 H ㎜, CLEA Japan Inc., Tokyo). The bedding materials were obtained from a neighborhood joiner and wood shaving chips were sifted with a separator machine (TK-30VS, Tamiya Inc., Tokyo) so that the size of chips was 10～2.4 ㎟. All these residence materials were used after being autoclaved.

Experimental design

Rats were raised in one of 4 different conditions: they were housed individually (I-WMC) or in a group of 3 rats (G-WMC) in wire mesh cages, or individually (I-PWB) or in a group of 3 rats (G-PWB) in plastic cages with wood shaving chips. During the experimental period, animals were fed on a plain commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo) and 1% NaCl water *ad libitum*. The water consumption, food consumption and body weight were checked at 0, 4, 6, and 8 weeks. Systolic blood pressure (SBP) was measured by tail-cuff method (UR-1000; Ueda Electric Works, Tokyo, Japan) every 4 weeks until 12 weeks (6). Animals were observed daily for neurological symptoms. The definition of neurological symptomatology has been described by Yamori's studies (7). Briefly, abnormal symptoms suggestive of stroke or hypertensive encephalopathy were excitement (piloerection, hyperkinesis), hyperirritability (jumping, escaping), paroxysm (aggressiveness, rage, fighting), behavioral and psychological depression (hypokinesis, hyposthenia, hypotonia, hyporesponsiveness), and motion disturbance (repetitive lifting of paws, ataxia, paresis, paralysis). No quantitative evaluation of the individual symptoms was remarked. Late symptoms observed near the time of death were apathy, coma, urinary incontinence, and other rarer symptoms (hyperphagia, self-biting). Rats were autopsied soon after natural death, and the brain, heart, kidneys, and adrenals were harvested. All experiments were performed according to the animal experiment guidelines of Shimane Medical University.

Statistical analyses

Data were expressed as means ± standard error of the mean (SEM). Statistical analysis was performed by a two-way analysis of variance (ANOVA). When F value was significant, Fisher's PLSD test was used as a *post-hoc* test to determine significant differences in multiple comparisons. A value of $p<0.05$ was taken as statistically significant.

RESULTS

1. Survival rate

Figure 1 shows that the survival rate of the I-WMC rats was 100% until 4 weeks, but all died by 7 weeks. The survival rate of the G-WMC rats was 100% at 5 weeks, and decreased gradually thereafter by 10 weeks. The survival rate of the I-PWB rats was 100% at 8 weeks, and decreased gradually thereafter until 17 weeks. The survival rate of the G-PWB rats was 100% at 10 weeks. Thereafter, by the end of 24 weeks, all animals died. The 50% survival of the I-WMC rats and the G-WMC rats were attained about 6 and 7 weeks, respectively, and those of the I-PWB rats and the G-PWB rats were attained at about 10 and 12 weeks, respectively. The survival rate was significantly different between the two residential conditions (WMC vs. PWB).

Average life-span in the I-PWB rats (82 ± 9 days) was significantly longer than that of the I-

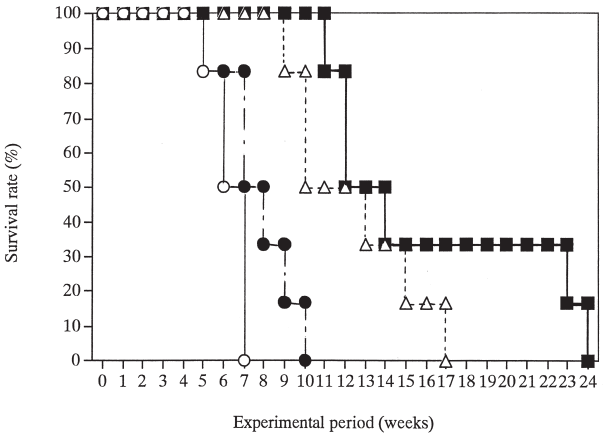

Fig. 1. Effects of residential condition and population density on the survival rate of the SHRSP given 1% salt water for drinking.
○ I-WMC, ● G-WMC, △ I-PWB, ■ G-PWB

Table 1. Comparison of life-span and incidence of stroke in SHRSP

Groups	Number of rats	Life-span (days)	Lifetime after start of experiment (days)	Incidence time of stroke (days)
I-WMC	6	114 ± 2	39 ± 2	31 ± 2
G-WMC	6	123 ± 4	51 ± 4	40 ± 4
I-PWB	6	156 ± 10	82 ± 9	60 ± 8
G-PWB	6	182 ± 16	108 ± 16	80 ± 11

Results are expressed as the mean ± SEM for 6 animals. Significant difference at **, P<0.01; ***, P<0.001

WMC rats (39 ± 2 days). Similarly, average life-span of the G-PWB rats (108 ± 16 days) was significantly longer than that of the G-WMC rats (51 ± 4 days). Average life-span of the G-PWB rats was slightly longer than that of the I-PWB rats. Average life-span was not different between the two groups in the WMC (Table 1).

2. Time to incidence of stroke

Abnormal symptoms suggestive of stroke or hypertensive encephalopathy were observed in all of 24 rats which were confirmed to have cerebral lesions at autopsy (Table 1). The development of stroke was significantly earlier in the I-WMC rats (31 ± 2 days) than in the I-PWB rats (60 ± 8 days). The onset of stroke in the G-WMC rats (40 ± 4 days) was significantly earlier than that of the G-PWB rats (80 ± 11 days).

3. Body weight

Body weight changes in each group are shown in Fig. 2. Until 4 weeks, there were no significant differences in the growth among the four groups. However, the I-WMC rats and the G-WMC rats became moribund and weak from 5 weeks (data not shown). In the G-WMC rats, body weight was lost from 6 to 8 weeks (from 270 ±18 g to 228 ±4 g).

4. Water consumption

Figure 3 shows that all groups showed an increase in water consumption during the first 4 weeks. Water consumption of the G-WMC rats was significantly greater than that of the G-PWB rats at 4 and 6 weeks. Water consumption of the G-WMC rats was significantly greater than that of the I-WMC rats at 6 weeks.

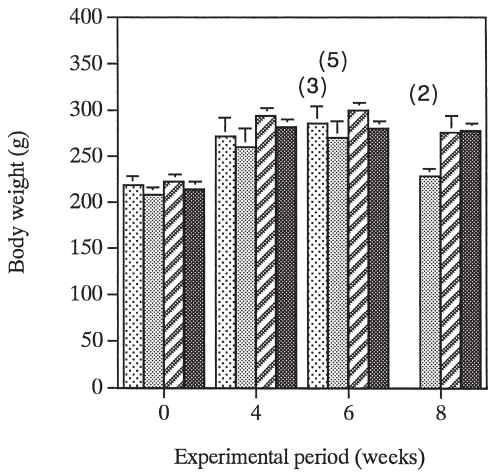

Fig. 2. Effects of residential condition and population density on body weight of the SHRSP. Data are presented as mean+SEM. ( ), Number of rats examined; the other groups included 6 animals.
I-WMC, G-WMC, I-PWB, G-PWB

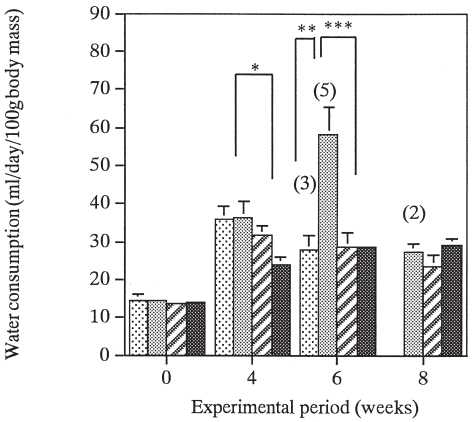

Fig.3. Effects of residential condition and population density on water consumption of the SHRSP given 1% salt water for drinking. Data are presented as mean+SEM. Significant differences from each value. *, p<0.05; **, p<0.01; ***, p<0.001. ( ), Number of rats examined; the other groups included 6 animals.
I-WMC, G-WMC, I-PWB, G-PWB

5. Food consumption

Changes in food consumption of each group are shown in Fig. 4. No major difference was seen in food consumption among the four groups until 4

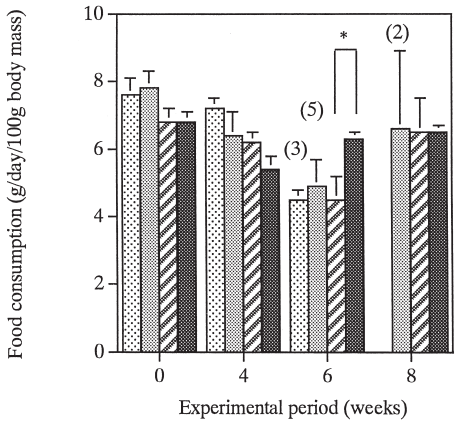

Fig. 4. Effects of residential condition and population density on food consumption of the SHRSP. Data are presented as mean+SEM. Significant differences from each value. *, $p<0.05$. ( ), Number of rats examined; the other groups included 6 animals.
I-WMC, G-WMC, I-PWB, G-PWB

weeks. And the G-PWB rats showed significant higher consumption than that of the I-PWB rats at 6 weeks.

6. *Blood pressure*

Figure 5 illustrates that all rats showed an increase in SBP during the first 4 weeks although the grade of hypertension was not significantly different. The SBP difference between the I-PWB and the G-PWB groups were not significant at 12 weeks.

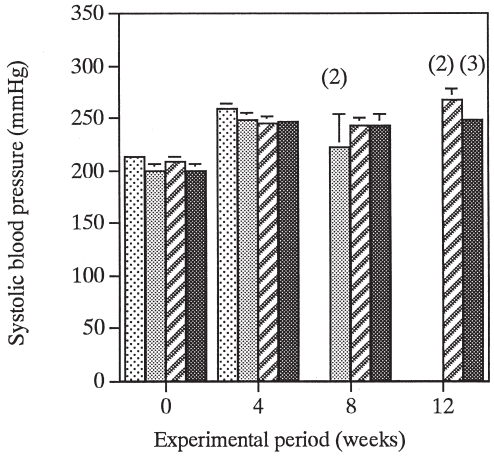

Fig. 5. Effects of residential condition and population density on systolic arterial blood pressure of the SHRSP. Data are presented as mean+SEM. ( ), Number of rats examined; the other groups included 6 animals.
I-WMC, G-WMC, I-PWB, G-PWB

7. Organ weight

Table 2 shows that brain weights in the individually-housed and those housed in a group of 3 rats were significantly heavier in the PWB rats than in the WMC rats. The final incidences of stroke in all groups were 100%. All cerebral stroke lesions were the softening caused by infarction. Weights of the heart, kidneys and adrenals were not different among the four groups.

DISCUSSION

It is noteworthy that the average life-span was significantly extended in the rats bred in the PWB than that in the WMC. In spite of salt-loading, the rats bred in the PWB residence materials delayed the development of severe hypertension and the incidence of stroke. The PWB residence materials would be better for keeping warm and having good elasticity than the WMC residence materials (8). Namely, the WMC residence materials have the high thermal conductivity and promote heat dissipation (9). The rats bred in the WMC residence materials were restricted from scooping out a hole (10) or from scratching sawdust with their forefeet (8). Thus, the rats in the WMC condition might be loaded with emotional or psychosocial stress. Residence materials have an impact on the health and well-being of the animals and may cause biased experimental results. Manser *et al.* (11) reported that the rats preferred the wood-shaving chips rather than the wire-mesh, by the method of two-preference test. There was a marked difference in the preference for the solid floor, depending upon whether rats were active or resting. Similarly, Blom *et al.* (10) using rats and mice also found that wood-chip bedding was the most favorite of five different types of bedding materials. The preference of rats and mice for wood-shaving chips is in accordance with our present results. As bedding is an environmental factor which is permanently present during the laboratory rodent's lifetime, the type of residence may influence its welfare (11, 12).

When rats are housed in groups, they clearly delayed the development of severe hypertension and the incidence of stroke. Klir *et al.* (13) measured water and food intake and organ weights of rats housed in 874 cm^2 cages in a group of 1, 2, 4, 6 or 8. They found that those housed 3 or 4 to a cage weighed the most in the groups. In addition, the isolation caused loneliness feeling which was incurable

Table 2. Organ weights of four groups

Groups	Number of rats	Body weight (g)	Brain (g)		Heart (g)	Kidneys (g)	Adrenals (mg)
I-WMC	6	217.3 ± 12.9	2.05 ± 0.04	**	1.39 ± 0.09	2.85 ± 0.17	64.73 ± 6.27
G-WMC	6	200.7 ± 23.4	2.14 ± 0.05		1.19 ± 0.14	2.63 ± 0.18	62.80 ± 3.61
I-PWB	6	204.5 ± 12.3	2.44 ± 0.08	*	1.36 ± 0.09	3.11 ± 0.20	61.93 ± 7.39
G-PWB	6	226.2 ± 10.4	2.47 ± 0.13		1.51 ± 0.13	2.92 ± 0.11	72.73 ± 3.77

Results are expressed as the mean ± SEM for 6 animals. Significant difference at *, $P<0.05$; **, $P<0.01$.

(14, 15), increase in aggressiveness, and an escape behavior from contact with person. Thus, the stress might be accumulating even further (16, 17).

Salt-loading is known to accelerate the progress of hypertension in the SHRSP, and to cause the earlier occurrence of cerebrovascular lesions (stroke) (2, 4, 18). Isolation caused emotional stress resulting in the increased water intake, which lead to death from stroke through the rapid development of severe hypertension. Similar experiment showed that isolated individuals drank significantly more alcohol than the group-housed rats (19). Thus, the excessive water consumption in isolated rats suggests a feeling of loneliness. As to organ weights, the weight of brain was significantly greater in the PWB conditions than in the WMC conditions. The difference may depend on the length of life-span rather than on a difference in residence material.

Our experimental data clearly showed that the residential condition for rats influenced the development of stroke and survival rate in the SHRSP given 1% NaCl solution for drinking, and suggested further that the environmental conditions, in association with emotional or psychosocial stress, might affect the development of hypertension-related cardiovascular disease. At this moment, there is an increasing requirement for disease model animals. Therefore, numerous studies have been done and yet ongoing as to the genomics and genetic analyses of the animal models for human diseases. However, just few studies have been done about environmental conditions that surround experimental animals. When we carry out animal breeding of disease model, it is internationally needed to standardize a breeding appliance and quality. To obtain experimental results of substantially high reproducibility and reliability, we should keep it in minds to minimize the influence of environmental factors upon results from animal experiments. For future studies, our present study may provide some suggestions to this goal.

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