REDUCED SODIUM EXCRETORY ABILITY IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS

Akinobu NAGAOKA, Mitsuru KAKIHANA, Masaki SHIBOTA, Kazuo FUJIWARA and Kozo SHIMAKAWA
Biology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka 532, Japan
Accepted May 17, 1982

Abstract—The excretory response of spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) to acute saline load was compared to that of control Wistar Kyoto rats (WKY) in pre- and early-hypertensive phases. In each of the 3 groups, half of the animals was fed a low salt diet, and the other half a normal diet. At the prehypertensive phase, sodium and water excretion and sodium-potassium ratio in the urine in SHR and SHRSP fed a normal diet were significantly less than those in WKY. The ability of SHR to excrete sodium and water, however, was improved by the elevation of blood pressure that developed between 7 and 10 weeks after their birth. While young SHR fed a normal diet had a reduced ability to excrete sodium, the young SHR fed a sodium-restricted diet did not. Salt restriction significantly delayed the appearance of high blood pressure in both SHR and SHRSP. These results suggest that in both SHR and SHRSP, an elevation of blood pressure is important to compensate for the reduced ability to excrete sodium and water.

Spontaneously hypertensive rats (SHR) have been widely used as an experimental model for human essential hypertension. A stroke-prone strain of SHR (SHRSP), established by Okamoto et al. (1) in 1974, shows a more abrupt rise of blood pressure than SHR, especially at a young age (2, 3). Recent studies have indicated that the kidney is a pathogenetic factor in the hypertension of SHR (4-6) and SHRSP (7). In a previous study (2), we demonstrated that both SHR and SHRSP were very sensitive to salt, showing a further increase of blood pressure in addition to the spontaneous increase of blood pressure. Control Wistar Kyoto rats (WKY) did not respond to salt, but the F1 hybrids between SHR and WKY showed an intermediate response. These results suggest that the increased sodium sensitivity is one of the genetic characteristics of SHR. More recently, it was hypothesized that kidneys of SHR and SHRSP require a higher arterial pressure than kidneys of WKY to excrete a given amount of salt and water (4, 8). All these findings indicate that sodium and water excretory ability may be altered in the SHR, leading to the development of hypertension. On the other hand, it has been reported that the ability of young (6-9 week old) SHR to excrete sodium is equal to (9) or lower (10-12) than that in control Wistar rats under anesthetic conditions and that sodium balance is normal in SHR (13, 14) and positive in SHRSP (13-15) in the pre- and early-phases of hypertension.

In the present study, the ability of young, conscious SHR and SHRSP to excrete an acutely administered saline load was ex-
amiined in order to inquire further into the pathogenetic relationship of renal sodium handling to spontaneous hypertension. In addition, the effects of salt-intake restriction on renal sodium handling and the development of hypertension were investigated in both SHR and SHRSP.

MATERIALS AND METHODS

Male stroke-prone SHR (SHRSP), stroke-resistant SHR (SHR), and control WKY were 4 weeks of age at the start of the experiments. The source of the rats has been reported elsewhere (1, 2). SHR and SHRSP were in the F53 and F54 generations, respectively. All of the animals were weaned at 27 or 28 days after birth, housed in separate cages, and fed a synthetic diet (sucrose, 67.6%; casein, 18.0%; cotton seed oil, 8.0%; salt mixture, 4.0%; cellulose powder, 1.5%; vitamin mixture, 0.5%; dl-methionine, 0.3%; and choline chloride, 0.1%). At 32 days of age, each strain of rats (n=12) was divided into two groups. One group was maintained on a normal sodium diet (11.1 meq/100 g diet) and the other on a sodium restricted diet (1.11 meq/100 g diet). Potassium content was 15.7 meq/100 g diet in both groups.

Systolic blood pressure in the tail artery was measured by the pulse-pick up method (2) at 4, 5, 7, and 9 weeks of age. At the end of the experimental period (10 weeks of age), the mean arterial pressure in the femoral artery was measured directly using a transducer and polygraph 3 hr after the insertion of an arterial cannula. The cannulation was performed under ether anesthesia. Two days after each measurement of systolic blood pressure, the rats were given orally a volume of 0.9% saline equivalent to 2.5% of the body wt. The rats were fasted 20 hr prior to this sodium load, but were allowed free access to distilled water. After the saline loading, 3-hr (10:00–10:30 to 13:00–13:30) urine was collected using small isolation cages whose floors were covered with plastic sheets. The rats were moved into the urine-collecting cages one hr before the saline loading to allow them to adapt. The marks of urine droplets were rinsed with a small volume of distilled water. At the end of the collecting period, the animals were placed in an ether inhalation box to accelerate discharge of urine remaining in the bladder. In a preliminary study, we found this procedure was effective. Urinary volume and sodium and potassium excretion were measured. The metabolic experiments were done on Wednesday for normal diet groups and on Thursday for low sodium diet groups. Arterial blood samples were also taken through the arterial cannula at the termination of the experiments. Sodium and potassium concentrations in urine and plasma were analyzed using flame photometry (Corning 455).

Data were expressed as the means±S.E., and statistical analysis was performed with the Student’s t-test for unpaired groups. P<0.05 was considered statistically significant.

RESULTS

Initial blood pressure, and sodium and water excretion in WKY, SHR, and SHRSP (values at 4 weeks of age): Data are summarized in Table 1. Systolic blood pressure in immature (4 weeks of age) SHR and SHRSP was in the normotensive ranges, but significantly higher than that in WKY. Urinary volume and sodium excretion in 3-hr urine samples were significantly less in the two strains of SHR than in control WKY; the values in SHR and SHRSP were not significantly different. Potassium excretion was similar in the three strains (102–109 meq per 100 g body wt.), and as a consequence, the sodium-potassium ratio in SHR (1.50±0.11) and SHRSP (1.50±0.09) was significantly


lower than that in WKY (2.74±0.15). Body wt. in SHRSP was significantly less than that in WKY and SHR.

**Age-related changes in sodium and water excretion in normal and sodium restricted groups in WKY, SHR, and SHRSP:** Data are shown in Table 2. The rats in each strain were divided into normal and low sodium diet groups after obtaining the urinary sodium and water excretion values at 4 weeks of age. In the normal sodium groups, sodium excretion at 4 and 5 weeks of age in both SHR and SHRSP was significantly less than that in WKY. At 7 weeks of age, a significant difference between hypertensive and control rats was observed in sodium excretion but not in urinary output. At 10 weeks of age, there were no differences among the 3 strains in

| Table 1. Initial values of urinary output, sodium excretion, and blood pressure in Wistar Kyoto rats (WKY), spontaneously hypertensive rats (SHR), and stroke-prone SHR (SHRSP) |
|-----------------------------------------------|
| **Urinary output (ml/100 g body wt.)** | **WKY (n=12)** | **SHR (n=12)** | **SHRSP (n=12)** |
| Sodium excretion (µeq/100 g body wt.) | 1.46±.10 | 1.18±.07* | 0.89±.07* |
| Systolic blood pressure (mmHg) | 292±11 | 144±7* | 154±9* |
| Body weight (g) | 92±.1 | 108±1* | 102±1* |

The animals were loaded with a volume of saline equivalent to 2.5% of the body wt. (386 µeq of sodium per 100 g body wt.). *P<0.05. Mean±S.E. Age: 4 weeks old.

| Table 2. Age-related changes in sodium and water excretion in WKY, SHR, and SHRSP fed on normal and low sodium diets |
|-----------------------------------------------|
| **4 week old** | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) |
| WKY | 1.53±.15 | 299±12 | 1.39±.15 | 284±19 |
| SHR | 1.19±.11 | 140±10* | 1.17±.10 | 143±10* |
| SHRSP | 0.81±.09* | 156±9* | 0.98±.13 | 151±17* |

On normal diet

| **5 week old** | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) |
| WKY | 1.76±.05 | 337±13 | 0.56±.06* | 11±3* |
| SHR | 0.89±.12* | 168±12* | 0.61±.08 | 11±7* |
| SHRSP | 0.74±.12* | 164±22* | 0.57±.07 | 47±10** |

On low sodium diet

| **7 week old** | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) |
| WKY | 1.37±.09 | 215±10 | 0.82±.11* | 88±23* |
| SHR | 1.10±.12 | 140±18* | 0.77±.10 | 37±19* |
| SHRSP | 1.14±.15 | 155±11* | 0.73±.12 | 49±13* |

| **10 week old** | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) | **UV** (ml/100 g BW) | **UnV** (µeq/100 g BW) |
| WKY | 1.25±.15 | 122±12 | 0.79±.11* | 75±11* |
| SHR | 1.24±.12 | 110±22 | 0.79±.10 | 79±14 |
| SHRSP | 0.95±.11 | 128±10 | 1.10±.17 | 118±14* |

UV, urinary volume; UnV, sodium excretion; BW, body weight. *P<0.05 vs WKY and *P<0.05 vs the normal sodium group. n=6. Mean±S.E.
urinary and sodium excretion. The sodium-potassium ratio in urine in SHR (1.48±0.12) and SHRSP (1.71±0.09) at 4 weeks of age was significantly lower than that in WKY (2.97±0.21).

In each strain, sodium and water excretion in the sodium restricted groups was markedly less than that in the groups fed on a normal diet; the difference was largest in WKY. In each strain, the effect of sodium restriction on sodium and water excretion tended to be lessened as the period of sodium restriction increased. Under sodium restricted conditions, there was no strain difference in the sodium excretion between control and hypertensive rats except for 5 and 10 week old WKY and SHRSP; SHRSP excreted more sodium. Sodium restriction did not affect body weight gain in the three strains; body wt. in 10 week old WKY, SHR, and SHRSP were 264±6, 277±6, and 231±5 g in the normal sodium groups and 259±4, 283±11, and 233±3 g in the sodium restricted groups, respectively.

There were no significant differences in plasma sodium (144.5-146.7 meq/l) and potassium (3.50-3.89 meq/l) concentrations among the six groups.

Age-related change in blood pressure and the effect of sodium restriction: The results are illustrated in Fig. 1. Blood pressure in groups fed the normal diet was elevated age-dependently. However, blood pressure in WKY did not rise over 120 mmHg. Blood pressure in SHR and SHRSP at 4 and 5 weeks of age was significantly (P<0.05) higher than that in WKY, although it was within the normal range. At 7 to 10 weeks of age, both strains of SHR showed markedly higher blood pressure than WKY. The pressure rose over 150 mmHg after 9 weeks of age in SHR and at around 7 weeks of age in SHRSP.

Sodium restriction significantly depressed the elevation of blood pressure in SHR and SHRSP. There was a significant difference between the normal and sodium restricted groups of both hypertensive rats after 7 weeks of age (Fig. 1). In WKY, the sodium restricted group showed a slightly (P>0.05) lower blood pressure than the normal group.

DISCUSSION

Since the experiments in this study were performed on conscious animals, a methodological problem should be considered, namely, whether the urine was completely sampled throughout the experimental period. To resolve this issue, a separate group of 5 week old WKY (n=7) and SHRSP (n=7) was killed at the end of the urine collection period, and the urine retained in their bladders was examined; the volume of the retained urine was negligible. The results on urine and sodium excretion were similar to those described above. For example, sodium excretions in WKY and SHRSP were 292±21 and 189±21 meq/100 g body wt., respectively; and the difference was statistically significant.

Young SHR and SHRSP fed the normal diet had reduced ability to excrete sodium.
In particular, at 4 weeks of age, the blood pressure in SHR and SHRSP was within the normal range; but the urinary sodium and water excretion and the sodium-potassium ratio in SHR and SHRSP were significantly lower than those in WKY. Similar results were demonstrated in 5 week old animals, except for the urinary sodium-potassium ratio. The reduced ability of SHR and SHRSP to excrete sodium and water was, however, improved with the elevation of blood pressure. These data, especially those on SHRSP, support previous results by others (13–15). According to our results (8) and those of Arendshorst and Beierwaltes (4), when renal perfusion pressure in adult SHR and SHRSP is reduced to a level similar to that in WKY, urinary sodium and water excretion are markedly decreased. These findings strongly suggest that the reduced sodium excretory ability is a primary defect in the SHR and SHRSP and that the elevation of blood pressure (renal perfusion pressure) may compensate for the reduction of the ability to excrete sodium in both hypertensive rats.

Recently, Grim et al. (16) have assessed the influence of heredity on factors that help regulate the arterial blood pressure in humans. They observed sluggish natriuretic responses in relatives of essential hypertensive patients. This observation and the results of the present study suggest a heritable influence on renal sodium handling, supporting the hypothesis that the decreased sodium excretion may be one of the genetic characteristics of SHR.

Sodium restriction resulted in a marked decrease in sodium and water excretory responses in each strain that tended to be recovered gradually as the sodium restriction was prolonged. The recovery seems to be due to homeostatic responses such as activation in the renin-angiotensin-aldosterone system. Under conditions of sodium restriction, the strain differences in sodium and water excretion were not observed constantly. Sodium and water excretion in SHR were similar to those in WKY, and sodium excretion in SHRSP was more than that in WKY only at 5 and 10 weeks. This increase at 10 weeks is likely to be the "exaggerated natriuresis" of hypertension. Although the exact mechanism of the increased sodium excretion in 5 week old SHRSP is unknown, a possible explanation is that SHRSP rats may have higher adaptability to the sodium restriction through the homeostatic and/or other factors involved in sodium reabsorption.

The development of hypertension was significantly depressed in SHR and SHRSP fed a low sodium diet. The data clearly indicate that the reduced ability of the two strains of SHR to excrete sodium and water is involved in the genesis of spontaneous hypertension. However, the effect of sodium restriction on the development of hypertension was partial. These findings suggest that dietary sodium (even normal concentrations of sodium) seems to be, in part, related to the pathogenesis of hypertension in the SHR with reduced ability to excrete sodium and water.

In conclusion, the present study suggests that the ability of the SHR and SHRSP to excrete sodium and water is reduced in the prehypertensive phase, and it is likely that an increase in blood pressure will compensate for the reduced ability of SHR and SHRSP to excrete sodium and water.

Acknowledgements: We thank Dr. J.R. Miller for revision of the manuscript and K. Hamajo for excellent technical assistance.

REFERENCES
1) Okamoto, K., Yamori, Y. and Nagaoka, A.: Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circulation Res. 34 and 35, Supp. 1, 143–153 (1974)
2) Nagaoka, A., Iwatsuka, H., Suzuki, Z. and Okamoto, K.: Genetic predisposition to stroke in spontaneously hypertensive rats. Am. J. Physiol. 230, 1354–1359 (1976)
3) Nagaoka, A. and Lovenberg, W.: Plasma nor-
epinephrine and dopamine-β-hydroxylase in
 genetic hypertensive rats. Life Sci. 19, 29–34
 (1976)

4) Arendshorst, W.J. and Beierwaltes, W.H.: 
Renal tubular reabsorption in spontaneously
 hypertensive rats. Am. J. Physiol. 237, F38–
 F47 (1979)

5) Evan, A.P., Luft, F.C., Gattone, V., Connors,
 B.A., McCarron, D.A. and Willis, L.R.: The
 glomerular filtration barrier in the spontaneously
 hypertensive rats. Hypertension 3, Supp. I,
 154–161 (1981)

6) Kawabe, K., Watanabe, T.X., Shiono, K. and
 Sokabe, H.: Influence on blood pressure of renal
 isografts between spontaneously hypertensive
 and normotensive rats, utilizing the F₁ hybrids.
 Japan. Heart J. 19, 886–894 (1978)

7) Dietz, R., Schomig, A., Haebara, H., Mann,
 J.F.H., Rascher, W., Luth, J.B., Grünherz, N.
 and Gross, F.: Studies on the pathogenesis of
 spontaneous hypertension of rats. Circulation
 Res. 43, Supp. I, 98–105 (1978)

8) Nagaoka, A., Kakihana, M., Suno, M. and
 Hamajo, K.: Renal hemodynamics and sodium
 excretion in stroke-prone spontaneously hyper-
 tensive rats. Am. J. Physiol. 241, F244–F249
 (1981)

9) Mullins, M.M. and Banks, R.O.: Age-related
 changes in Na excretion in saline-loaded
 spontaneously hypertensive rats. Am. J. Physiol.
 231, 1364–1370 (1976)

10) Farman, N. and Bonvalet, J.P.: Abnormal
 relationship between sodium excretion and
 hypertension in spontaneously hypertensive
 rats. Pflügers Arch. 354, 39–53 (1975)

11) Takagi, M., Naknishi, N., Kubo, K., Muto, H.,
 Takuma, T. and Sugino, N.: Sodium metabolism
 in SHR—Effects of saline infusion on renal
 sodium excretion (abstract). Japan. Heart J.
 22, 467 (1981)

12) Shiigai, T., Tomita, K., Shinohara, S., lino, Y.,
 Saito, H., Matsuda, O. and Takeuchi, J.: The
 effect of an acute increase in renal perfusion
 pressure on urinary sodium, water and kallikrein
 excretion in young SHR (abstract). Japan.
 Heart J. 22, 470 (1981)

13) Dietz, R., Haebara, H., Schomig, A., Rascher, W.,
 Berecek, K., Mann, J.F.E., Luth, J.B. and
 Gross, F.: The role of the kidney in the pathoge-
nesis of spontaneous hypertension in rats.
 Japan. Heart J. 20, Supp. I, 52–54 (1978)

14) Yamori, Y., Horie, R., Ohtake, M., Nara, Y. and
 Ooshima, A.: Electrolyte balance in stroke-prone
 and -resistant SHR. Japan. Heart J. 20, Supp. I,
 65–67 (1978)

15) Ooshima, A., Horie, R., Komuro, T., Kitazawa, S.
 and Yamori, Y.: Possible involvement of altered
 electrolyte and water metabolism in the develop-
 ment of spontaneous hypertension in the rat.
 In The Kidney in Arterial Hypertension, Edited
 by Bianchi, B. and Bazzato, G., p. 119–126.
 Bunge, Utrecht (1979)

16) Grim, C.E., Luft, F.C., Miller, J.Z., Rose, R.J.,
 Christian, J.C. and Weinberger, M.H.: An
 approach to the evaluation of genetic influences
 on factors that regulate arterial blood pressure
 in man. Hypertension 2, Supp. I, 34–42 (1980)