PARABIOTIC EFFECT ON BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS

YUKIO YAMORI, M. D., D. M. SC.

No significant elevation of blood pressure was observed in control Wistar rats parabiotically united with spontaneously hypertensive rats (SHR). SHR showed a slight lowering of blood pressure when they were united with control Wistar rats, but such a lowering effect was not obvious in SHR united with unilaterally nephrectomized Wistar rats. The blood pressure of the partner of SHR parabiotics maintained hypertension for 10 days after bilateral nephrectomy. These results showed that SHR had no humoral transmissible pressor substance and that the kidney of SHR had no positive role in maintaining hypertension.

PARABIOSIS is a classical technique used for the study on the humoral transmission of various substances. In this study parabiosis was applied in the spontaneously hypertensive rats (*Okamoto* and *Aoki*) (SHR)¹⁻³ to explore possible humoral factors in the pathogenesis of spontaneous hypertension and to clarify the role of kidney in this genetic hypertension. The SHR is now established as an inbred strain after successive brother-sister matings and is apparently an adequate model for the study on hypertension. As for the pathogenesis many studies have been done, and both neural^{2,4} and humoral^{2,5} factors are conceivable, but no conclusion has yet been obtained on the role of humoral factors in this hypertension.

One limitation of parabiotic experiments when applied to detect the humoral pressor factors, is parabiotic hypertension^{6,7}. However, parabiotic hypertension can be differentiated from other types of hypertension by the administration of

cortisone, for it is completely suppressed by daily administration of cortisone⁸. On the other hand, the effect of humoral pressor substances in SHR, if any, is not abolished by the cortisone treatment but rather intensified. Cortisone enhances the development of spontaneous hypertension⁹ or restores the hypertension in adrenalectomized SHR¹⁰. Therefore, the parabiotic effect on blood pressure was observed in parabionts which had been treated with cortisone in this experiments.

MATERIALS AND METHODS

The F₁₉₋₂₀ generation of spontaneously hypertensive rats¹⁻³ in NIH (SHR/N)¹¹ and control Wistar rats of NIH (N) or of Zivic-Miller Lab. P. A. (W) were used in this experiment. They were maintained on a standard Purina chow (5010) and distilled water for the experimental period.

The blood pressure was usually measured weekly without anesthesia by a tail-plethysmographic method^{1,12}. A special holder to accommodate parabiotic rats was used to measure blood pressure in them. Blood pressures are reported in mmHg.

Key Words: Hypertension
Spontaneously Hypertensive Rat
Parabiosis
Cortisone
Nephrectomy

(Received for publication, May 4, 1971)

Department of Pathology Faculty of Medicine, Kyoto University, Kyoto

* This work was done at Experimental Therapeutics Branch, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland, U.S.A. when the author was visiting scientist there.

Parabiosis was made under sodium pentobarbital anesthesia (30 mg/kg) according to the technique of *Bunste and Meyer*,¹³ but the following procedures were modified. 1) Both ventral abdominal walls of the parabiotic pairs were sutured at first, and when the dorsal walls were united with each other continuously with no. 00 cut gut, the four edges of the abdominal walls were sutured together only at 3 or 4 points to keep the anastomosis through small holes between two abdominal cavities of the pairs. This caution was observed to prevent the strangulation of the intestine due to the displacement through a large anastomosis. 2) Not only the scapulas but also iliac bones of both animals were sutured to confirm the connection. The tails of the parabiotic pairs were bound to each other with adhesive tape for 2 weeks after the operation. All the rats were treated daily with Penicillin G (about 10,000–12,000 I.U.) for 2 days before and for 2 weeks after the operation. In the nonparabiotic sham-operated rats skin and abdominal walls were incised and sutured.

In the first experiment, 19 male SHR's at the age of 60–70 days and 29 age-matched male Wistar rats of Zivic-Miller Lab. (W) were used. Among them parabiosis was made between SHR and W (S-W), between 2 SHR's (S-S), and between 2 W's (W-W). The number of the parabiotic pairs and sham-operated rats in excellent physical condition after operation were as follows: 10 W-W, 5 S-W, and 4 sham-operated SHR's and W's, respectively. The W-W parabionts were treated with 1 mg/day (s.c.) of cortisone acetate (Cortone, Merck) for the fourth and the fifth weeks to eliminate the parabiotic hypertension.^{6–8} The S-S, and S-W parabionts were also treated with the same dose of cortisone acetate for the fifth week.

In the second experiment, 23 female SHR's (S) of 60–70 days of age and 9 age-matched female NIH Wistar rats (N) were used. Unilateral nephrectomy was performed in N (N_R) united with SHR and in SHR (S_R) united with SHR when they were connected in parabiosis. The number of parabionts in excellent physical condition after the operation were as follows: 7 S_R -S, 4 N_R -S and 5 N-S. The unilaterally nephrectomized pairs of SHR parabionts were surgically deprived of the residual kidneys 1 month after the parabiosis and the blood pressure was observed daily after the bilateral nephrectomy.

The data on blood pressure was statistically analyzed by Student's small sample t test.

RESULTS

1. The Effect of Parabiosis on the Blood Pressure of Normotensive and Hypertensive Rats (Fig. 1)

The blood pressure in SHR united in parabiosis with control Wistar (W) was slightly lower 1 week after parabiosis, but not significantly different from that in SHR of other groups. SHR in all groups showed a similar lowering in blood pressure probably because of the postoperative condition. SHR in S-W parabionts showed a significantly lower blood pressure (163 ± 3) than nonparabiotic SHR (187 ± 4) or SHR in S-S parabionts (179 ± 4) 2 weeks after parabiosis. The significant difference continued

Fig. 1. Average blood pressure in parabiotic SHR and Wistar rats.

Average blood pressure ($M \pm SE$) of the following groups is shown; parabiosis between SHR and normotensive Wistar rats (S-W, $\circ$ - $\square$, solid circles show the significant decrease in blood pressure from that in parabiotic SHR or nonparabiotic SHR), SHR freed from parabiosis (Δ), parabiotic SHR (S-S, $\odot$), parabiotic Wistar (W-W, $\square$), one partner of these parabionts showed hypertension during the 3rd and 4th weeks and their average blood pressure is grouped separately as shown with the mark $\triangle$), and nonparabiotic SHR (Δ) and Wistar (∇). See the text for details.

thereafter. The blood pressures of SHR in S-W parabionts, of parabiotic SHR, and of nonparabiotic SHR were 171 ± 7 , 196 ± 10 and 194 ± 7 , respectively 4 weeks after parabiosis. At the fifth week of experiment 3 out of 5 S-W parabionts were freed from the parabiotic partners and the remaining parabionts were treated with daily doses of 1 mg cortisone acetate to eliminate the possibility of parabiotic hypertension. Although SHR in these S-W parabionts remained at a significantly lower blood pressure level (159 ± 3) than nonparabiotic SHR (205 ± 5) or SHR in S-S parabionts (196 ± 7), the SHR freed from the parabiosis with W showed an increase in blood pressure (205 ± 9). There was no significant difference between these SHR and nonparabiotic SHR or SHR in S-S parabionts.

On the other hand, W in S-W parabionts showed no significant difference in blood pressure from nonparabiotic W during the whole experimental period. The blood pressure in W-W parabionts was not significantly different from

W in other groups at the first and second weeks of experiment. However, one of each of these parabiotic pairs showed a sudden increase in blood pressure (176 ± 17) at the third week. They were treated with cortisone acetate (1 mg/day, s.c.) thereafter. The high blood pressure in these rats subsided gradually and there was no hypertension (133 ± 8) among them at the end of the experiment. While one partner in each W-W pair showed high blood pressure, the other partner exhibited normal blood pressure. The cortisone treatment did not affect the blood pressure in S-S parabionts.

These experiments indicated no pressor influence in W united with SHR but rather some antihypertensive influence in SHR united in parabiosis with W.

2. The Effect of Unilateral Nephrectomy in Normotensive Rats United with SHR on Blood Pressure (Fig. 2)

The second experiment confirmed no pressor influence in NIH Wistar rats (N) united with SHR and showed a similar lowering of blood pressure in SHR united with N to the effect observed in SHR partner of S-W parabionts in the first experiment. The blood pressure in N of S-N parabionts was 134 ± 5 and 133 ± 1 before and 1 month after operation, respectively. On the other hand, the blood pressure in SHR of S-N parabionts was 153 ± 2 one month after parabiosis while their preoperative level was 178 ± 4 .

Unilateral nephrectomy was performed in N and SHR when they were united with intact SHR. Even after unilateral nephrectomy no elevation of blood pressure was observed in these unilaterally nephrectomized N (N_R) united with SHR. Their preoperative and 1 month postoperative blood pressures were 137 ± 3 and 132 ± 4 , respectively. The blood pressure lowering effect observed in SHR united with intact N was not obvious in SHR united with N_R . The blood pressure in SHR of S- N_R parabionts was 168 ± 3 one month after parabiosis while their preoperative level was 172 ± 4 . This blood pressure was significantly higher ($p < 0.02$) than that (153 ± 2) in SHR united with intact N.

No enhancement of hypertension was observed in blood pressure in either partner of the parabionts between SHR and unilaterally nephrectomized SHR (S_R). The blood pressure in SHR of S- S_R parabionts (174 ± 2) was significantly higher ($p < 0.001$) than that in SHR united with intact N.

This experiment indicated no pressor influence

Fig. 2. Unilateral nephrectomy in the partners of normotensive-hypertensive or hypertensive-hypertensive parabionts. Parabiosis was made between SHR (S) and NIH Wistar (N), between S and unilaterally nephrectomized N (S- N_R) and between S and unilaterally nephrectomized S (S- S_R). Unilateral nephrectomy was performed at the time of operation for parabiosis. Average blood pressure ($M \pm SE$) before and 1 month after parabiosis is shown in this simplified figure.

Fig. 3. Bilateral nephrectomy in one partner of the parabiotic SHR.

In one partner of unilaterally nephrectomized SHR (S_R) united with intact SHR (S), residual kidney was extirpated 1 month after unilateral nephrectomy and their average blood pressure ($M \pm SE$) is shown up to 10 days after bilateral nephrectomy.

even in N_R united with SHR and raised a possibility that the kidney in N had some antihypertensive influence while that in SHR had not.

3. The Effect of Bilateral Nephrectomy in One Partner of Parabiotic SHR on Blood Pressure (Fig. 3)

SHR were unilaterally nephrectomized and combined with intact SHR. There was no significant difference in blood pressure between SHR (175 ± 2) and unilaterally nephrectomized SHR (174 ± 4) 1 month after parabiosis. The remaining kidney in the unilaterally nephrectomized SHR was extirpated 1 month after parabiosis and the blood pressure was repeatedly checked thereafter. Five out of 6 pairs of these parabionts lived longer than 85 hours, although bilaterally nephrectomized SHR usually die within 48 hours.¹⁴ Four pairs survived for 166 hours after operation and 3 pairs of them remained longer than 10 days. The long survival of bilaterally nephrectomized partners in parabiosis showed that the connection between the parabiotic pairs was functional and allowed at least minimum exchange of extracellular fluids required to maintain the life of bilaterally nephrectomized rats.

There was no significant difference in the blood pressure between the bilaterally nephrectomized and intact SHR in the parabiotic unit. Their blood pressures were 169 ± 1 and 175 ± 5 , respectively, 166 hours after bilateral nephrectomy in one partner of the parabiotics. The bilaterally nephrectomized SHR were hypertensive (175 ± 1) for 10 days as long as they were observed after bilateral nephrectomy, and showed no great difference from the blood pressure (180 ± 2) in the intact partners.

This experiment showed that the kidney of SHR played no positive role in maintaining hypertension.

DISCUSSION

Dahl et al. separated two strains of rats which were sensitive or resistant to the development of hypertension following salt ingestion.^{15,16} They found that hypertension could be induced in the rat from the resistant strain when they were united in parabiosis with the sensitive strain and therefore speculated the presence of humoral transmissible influence from the sensitive strain.^{17,18} In contrast to *Dahl's* sensitive strain, spontaneously hypertensive rats (SHR)^{1-3,11} develop moderate to severe hypertension of over

200 mmHg uniformly without any treatment, and excess salt ingestion does not greatly affect the course of hypertension.¹⁹ Consequently, the genetic factors for hypertension or susceptibility to hypertension are different in SHR and *Dahl*'s strains. The recent detailed studies on the genetic mechanism of SHR showed that genetic determination of blood pressure was very high in SHR,²⁰ while the interaction of a nongenetic factor (NaCl) with the appropriate genetic substrate appeared to be necessary for the development of hypertension in *Dahl*'s sensitive strain.^{15,16} The present study of parabiosis supplied one more piece of evidence that the hypertension in SHR was greatly different from that in *Dahl*'s rats.

As the normotensive control Wistar united with SHR did not show any increase in blood pressure in this experiment, it was concluded that SHR had no humoral transmissible pressor substance. On the other hand, one partner of the paired SHR in parabiotic unit maintained high blood pressure after bilateral nephrectomy. Taken together, these results indicate that SHR's kidney has no positive role in the maintenance of hypertension. Up to the present, positive involvement of renin-angiotensin system in hypertension of SHR has been eliminated.^{21,22} An early study observed that unilateral or bilateral nephrectomy did not affect the level of hypertension in SHR.¹⁴ This early study of bilateral nephrectomy was incomplete because it was done without compensation of the excretory function of the extirpated kidneys. The present study on blood pressure effect of bilateral nephrectomy in parabiotic SHR supplements the previous study.

The blood pressure in SHR united with normotensive Wistar was slightly decreased, but the decrease was not apparent when the Wistar partner was unilaterally nephrectomized. It is, therefore, possible that normotensive rat's kidney might have some antihypertensive activity, which is partially or completely lacking in SHR's kidney. It is not clear whether this antihypertensive activity of the kidney in normotensive rats is physiological renal function, a depressor substance or the substance which inactivates a pressor material or suppresses its production. Many studies have been reported on the existence of renal depressor substances,²³⁻²⁸ but they have not yet been examined in SHR.

The Zymogram analysis of various organs from SHR demonstrated strain specific esterase iso-

zymes in the kidney of SHR and this biochemical abnormality was transmitted to the offspring in accordance with the law of co-dominancy.²⁹ This genetical-biochemical abnormality in SHR suggested that the renal function in SHR might be genetically different from that in normotensive rats. As genetic factors of the hypertension in SHR are possibly polygenic,^{20,30} a kind of renal functional abnormality in SHR might be one of the genetic factors.

SUMMARY

The possibility that humoral factors are involved in the hypertension observed in spontaneously hypertensive rats (SHR) was investigated by preparing various parabiotic pairs from SHR and normotensive rats of Wistar strain (NW). The blood pressure was measured by a tail-plethysmographic method under unanesthetized condition. Cortisone acetate (1 mg/day, s.c.) was administered on the 4th and 5th weeks after parabiosis to eliminate the parabiotic hypertension.

1. The blood pressure in normotensive rats united with SHR showed no significant elevation in comparison with that in parabiotic normotensive pairs or in nonparabiotic normotensives during the whole experimental period of 5 weeks. Their final blood pressures were 123 ± 9 , 133 ± 8 and 132 ± 4 mmHg, respectively.

2. The SHR united with NW showed a slightly lower blood pressure (171 ± 7) 4 weeks after parabiosis than either the parabiotic pairs of SHR (196 ± 10) or nonparabiotic SHR (194 ± 7). The blood pressure in the SHR united with NW increased to as high a level (205 ± 9) as in nonparabiotic SHR (205 ± 5) after they were freed from the parabiotic NW partners. This lowering of blood pressure observed in SHR united with NW was not obvious when SHR was united with unilaterally nephrectomized NW.

3. The blood pressure of one partner of SHR parabiotics maintained hypertension (175 ± 1) for 10 days after bilateral nephrectomy and showed no great difference from the blood pressure (180 ± 2) in their intact partners. The long survival of bilaterally nephrectomized partners of parabiotic pairs confirmed the functional connection between the pairs.

4. These results showed that SHR had no humoral transmissible pressor substance and that the kidney of SHR had no positive role in maintaining hypertension, and raised the possibility of a humoral antihypertensive mechanism in the

kidney of normotensive rats.

Acknowledgement

The author expresses cordial thanks to Drs. Albert Sjoerdsma and Walter Lovenberg, Laboratory Chief and Section Chief of Experimental Therapeutics Branch, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland, U.S.A. for their valuable advice and revision of this article.

REFERENCES

- OKAMOTO, K. & AOKI, K.: Development of a strain of spontaneously hypertensive rats. *Jap. Circulation J.* 27: 282, 1963.
- OKAMOTO, K.: Spontaneous hypertension in rats. *International Review of Experimental Pathology* 7: 227, 1969.
- OKAMOTO, K., TABEL, R., FUKUSHIMA, M., NOSAKA, S., YAMORI, Y., ICHIJIMA, K., HAEBARA, H., MATSUMOTO, M., MARUYAMA, T., SUZUKI, Y., & TAMEGAI, M.: Further observation of the development of a strain of spontaneously hypertensive rats. *Jap. Circulation J.* 30: 703, 1966.
- OKAMOTO, K., NOSAKA, S., YAMORI, Y., & MATSUMOTO, M.: Participation of neural factor in the pathogenesis of hypertension in the spontaneously hypertensive rat. *Japan Heart J.* 8: 168, 1967.
- AOKI, K., TANKAWA, H., FUJINAMI, T., MIYAZAKI, A., & HASHIMOTO, Y.: Pathological studies on the endocrine organs of the spontaneously hypertensive rats. *Japan Heart J.* 4: 426, 1963.
- HALL, C. E. & HALL, O.: Production and pathogenesis of parabiotic hypertension in the rat. *Arch. Pathol.* 51: 527, 1951.
- HALL, C. E. & HALL, O.: The relationship of sodium intake to the hypertensive hyalinosis syndrome produced in the rat by parabiosis. I. Hypertensive cardiovascular disease. *Texas Rep. Biol. Med.* 9: 714, 1951.
- HALL, C. E. & HALL, O.: The effect of cortisone on the course of parabiotic hypertension in the rat. *Endocrinol.* 50: 478, 1952.
- AOKI, K.: Experimental studies on the relationship between endocrine organs and hypertension in spontaneously hypertensive rats. II. Effects of various hormones on blood pressure. *Japan Heart J.* 4: 561, 1963.
- AOKI, K.: Experimental studies on the relationship between endocrine organs and hypertension in spontaneously hypertensive rats. III. Role of endocrine organs and hormones. *Japan Heart J.* 5: 57, 1964.
- YAMORI, Y., LOVENBERG, W., & SJOERDSMA, A.: Effect of 6 hydroxy-dopamine on blood pressure in spontaneously hypertensive and normotensive rats. *Europ. J. Pharmacol.* 13: 1971 (in press).
- WILLIAMS, J. R., HARRISON, T. R., & GROLLMAN, A.: A simple method for determining the systolic blood pressure of the unanesthetized rat. *J. Clin. Invest.* 18: 373, 1939.
- BUNSTER, E. & MEYER, R. K.: An improved method of parabiosis. *Anat. Rec.* 57: 339, 1933.
- AOKI, K.: Experimental studies on the relationship between endocrine organs and hypertension in spontaneously hypertensive rats. I. Effect of hypophysectomy, adrenalectomy, thyroidectomy, nephrectomy and sympathectomy on blood pressure. *Japan Heart J.* 4: 443, 1963.
- DAHL, L. K., HEINE, M., & TASSINARI, L.: Effects of chronic excess salt ingestion: Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. Exptl. Med.* 115: 1173, 1962.
- DAHL, L. K., HEINE, M., & TASSINARI, L.: Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature* 194: 480, 1962.
- DAHL, L. K., KNUDSEN, K. D., & HEINE, M.: Effects of chronic salt ingestion. Genetic influence on the development of salt hypertension in parabiotic rats: Evidence for a humoral factor. *J. Exptl. Med.* 126: 687, 1967.
- DAHL, L. K., KNUDSEN, K. D., & IWAI, J.: Humoral transmission of hypertension: Evidence from parabiosis. *Circulation Research* 24, 25, Suppl. 1: 21, 1969.
- LOUIS, W. J., TABEL, R., SPECTOR, S., & SJOERDSMA, A.: Studies on the spontaneously hypertensive rat: Genealogy, effects of varying salt intake and kinetics of catecholamine metabolism. *Circulation Research* 24, 25, Suppl. 1: 93, 1969.
- TANASE, H., SUZUKI, Y., OOSHIMA, A., YAMORI, Y., & OKAMOTO, K.: Genetic analysis of blood pressure in spontaneously hypertensive rats. *Jap. Circulation J.* 34: 1197, 1970.
- SOKABE, H.: Renin-angiotensin system in the spontaneously hypertensive rat. *Nature* 205: 90, 1965.
- HAGA, M., SOKABE, H., & OKAMOTO, K.: Juxtaglomerular cell granules in the spontaneously hypertensive rat. *Jap. Circulation J.* 30: 1479, 1966.
- GROLLMAN, A., WILLIAMS, J. R. Jr., & HARRISON, T. R.: The preparation of renal extract capable of reducing blood pressure of animals with experimental renal hypertension. *J. Biol. Chem.* 134: 115, 1940.
- PAGE, I. H., HELMAN, O. M., KOHLSTAEDT, K. G., FOUTS, P. J., & KEMPF, G. F.: Reduction of arterial blood pressure of hypertensive patients and animals with extracts of kidneys. *J. Exptl. Med.* 73: 7, 1941.
- MURIHEAD, E. E., JONES, F., & STIRMAN, J. A.: Antihypertensive property in renoprival hypertension of extract from renal medulla. *J. Lab. Clin. Med.* 56: 167, 1960.
- OKAMOTO, K., TAKEDA, T., TABEL, R., NOSAKA, S., & MATSUFUSE, R.: The depressor principle in the kidney of the newborn rat. I. Some biological and physiological properties of the kidney extract of the newborn rat. *Jap. Circulation J.* 28: 311, 1964.
- LEE, J. B., HICKLER, R. B., SARAVIS, C. A., & THORN, G. W.: Sustained depressor effect of renal medullary extract in the normotensive rat. *Circulation Research* 13: 359, 1963.
- LEE, J. B., COVINO, B. G., TAKMAN, B. H., &

- SMITH, E. R.: Renomedullary vasodepressor substance, medullin: Isolation, chemical characterization and physiological properties. *Circulation Research* 17: 57, 1965.
29. YAMORI, Y. & OKAMOTO, K.: Zymogram analysis of various organs from spontaneously hypertensive rats: A geneticobiochemical study. *Lab. Invest.* 22: 206, 1970.
30. LOUIS, W. J., TABEL, R., SJOERDSMA, A., & SPECTOR, S.: Inheritance of high blood pressure in the spontaneously hypertensive rat. *Lancet* 1: 1035, 1969.