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Spontaneous Hypertension Is Primarily the Result of Sympathetic Overactivity and Immunologic Dysfunction (42364)

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Abstract. Overactivity of the sympathetic nervous system and immunologic dysfunction have been shown to contribute to development and maintenance of hypertension in the Okamoto spontaneously hypertensive rat (SHR). In this study, the combined effects of reduction in sympathetic activity and immunologic manipulation on spontaneous hypertension have been determined. Neonatal SHRs received sham implants or implants of thymic tissue from Wistar donor rats. In addition, the thymus-implanted SHRs underwent bilateral renal denervation when they were 6 weeks old. At the same time, the sham-implanted SHRs underwent sham renal denervation. The denervations or sham operations were repeated when the SHRs were 9, 12, 15, and 18 weeks old. Wistar-Kyoto (WKY) rats also underwent serial sham renal denervations. Tail-cuff pressure measurements indicated that approximately 75% of the chronic hypertension in the SHRs was prevented by the combination of thymic implants and renal denervations. Direct arterial pressure measurements confirmed these results; when the rats were 21 weeks old, mean arterial pressure averaged 177 ± 5.5 mm Hg in sham-operated SHRs, 134 ± 2.7 mm Hg in implanted, denervated SHRs, and 121 ± 2.1 mm Hg in sham-operated WKY rats. These data indicate that overactivity of the sympathetic nervous system and immunologic dysfunction account for the majority of the hypertension in the Okamoto SHR.

Overactivity of the sympathetic nervous system contributes to the etiology of hypertension in the spontaneously hypertensive rat (SHR). Increased renal sympathetic nerve activity would be expected to be especially important in the development and maintenance of hypertension (1), and experimental evidence indicates that renal nerve activity is elevated in the SHR (2, 3). Also, it has been demonstrated that bilateral renal denervation delays the development of hypertension in the SHR (4). In addition, we (5) have determined that chronic ablation of the renal nerves produced by serial bilateral renal denervations not only delays development of the spontaneous hypertension, but also prevents full expression of hypertension in the SHR.

Dysfunction of the immune system in the SHR has also been implicated in the pathogenesis of spontaneous hypertension. The function of the T-lymphocyte system is abnormal in the SHR (6, 7), and several types of manipulations of the immune system in these rats exert an antihypertensive effect. Bendich *et al.* (6) have shown that short-term treatment of SHRs with antithymocyte serum will ameliorate the spontaneous hypertension. Also, we (8) have determined that chronic immunosuppressive treatment will delay the development of spontaneous hypertension and abolish about one-half of the hypertension chronically. Furthermore, attempts to correct the immunologic defect in the SHR by administration of thymic hormones (9, 10) or by implantation of thymic tissue from normotensive rats into the SHR (9, 11) have resulted in attenuation or prevention of the hypertension.

The purpose of the present study was to determine whether hypertension in the SHR could be prevented by chronic ablation of the renal nerves in combination with manipulation of the immune system in these rats. In previous experiments we have shown that chronic renal denervation will block approximately one-third of the expected progressive elevation of arterial pressure in the SHR (5) and that thymic implants from Wistar donor rats into neonatal SHRs will chronically prevent about one-half of the spontaneous hypertension (11). In this study we have combined neonatal thymic implants with chronic ablation of the renal nerves produced by serial bilateral renal denervations in the SHR. The

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arterial pressures of SHRs that have undergone these manipulations have been compared with the arterial pressures in sham-operated SHRs and sham-operated Wistar-Kyoto (WKY) control rats.

Materials and methods. *Thymic implants.* Neonatal thymic implants (n = 10) or sham implants (n = 10) were made in the male offspring of timed pregnant Okamoto SHRs (Taconic Farms, Germantown, N.Y.). As we have reported previously (11), serial thymic implants were given when the pups were 1, 8, and 15 days old. The implants consisted of freshly minced thymus gland (approximately 1- to 2-mm cubes) from 4- to 6-week-old male Wistar rats (Charles River Breeding Laboratories, Wilmington, Mass.). The 1-day-old SHR pups were anesthetized by hypothermia, and approximately one-fourth of a donor Wistar thymus gland was inserted subcutaneously with a pair of microforceps via a small skin incision in the inguinal region of the rats. The incision was closed with 1 or 2 7-O silk sutures, and the pups were rewarmed on a heating pad prior to returning them to their mothers. The sham-operated, 1-day-old SHR pups were anesthetized as described above and microforceps were used to probe the subcutaneous space via a similar skin incision. The SHR pups that received thymic implants or sham operations were selected at random in approximately equal numbers from each litter. Thymic implants or sham implants were repeated using similar techniques when the pups were 8 and 15 days old except that the older pups were anesthetized with methoxyflurane. Approximately one-third of a donor Wistar thymus gland was implanted into the 8-day-old SHRs, while one-half of a donor thymus gland was implanted into the 15-day-old rats.

The SHR pups were weaned when they were 4 weeks old. Tail-cuff pressures were monitored weekly in these 10 thymus-implanted and 10 sham-implanted SHRs once they were 6 weeks old, and, in addition, in 12 male WKY control rats (Taconic Farms). The weekly tail-cuff pressure for each rat was determined by averaging at least three readings obtained with a Narco Biosystems programmed electrosphygmomanometer.

Renal denervation. Following the measurement of control tail-cuff pressures when the rats were 6 weeks old, the 10 thymus-implanted SHRs underwent bilateral renal denervation. The 10 sham-implanted SHRs and 12 WKY rats underwent sham denervation procedures. The rats were anesthetized with pentobarbital sodium (50 mg/kg). Renal denervation was accomplished via a midline abdominal incision by bilaterally stripping the renal arteries and veins of all obvious nervous and connective tissue. These renal vessels were then painted with a solution of 10% phenol in ethanol. The sham-denervated rats received a similar midline abdominal incision, but the renal nerves were left intact. Therefore, rats were divided into three groups: sham-operated WKY rats, sham-operated SHRs, and thymus-implanted, renal-denervated SHRs.

As we have reported previously (5, 12, 13), the renal denervations (or sham denervations) were repeated at 3-week intervals to prevent possible regeneration of the renal nerves and return of functional renal nerve activity (14). The rats were anesthetized as described above when they were 9, 12, 15, and 18 weeks old and the renal denervations or sham denervations were repeated. During these successive bilateral renal denervations, all readily removable fibrous tissue was cleared away from the renal arteries and veins, and 10% phenol in ethanol was applied to these vessels for a few minutes with a fine brush.

Verification of pressure measurements and denervations. Tail-cuff pressure determinations were made weekly until the rats were 20 weeks old. When the three groups of rats were 21 weeks old they were anesthetized with pentobarbital sodium and polyethylene catheters (PE 90) with stretched tips were inserted into the lower abdominal aorta via the left femoral artery. These catheters were tunneled subcutaneously, exteriorized between the scapulae, and filled with heparin sodium (1000 U/ml). One to two days later the mean arterial pressure (MAP) was monitored in the conscious, unrestrained rats for a 1-hr period using Statham low-volume displacement pressure transducers in conjunction with a Grass polygraph.

Following the MAP measurements, the rats were sacrificed and the kidneys of the two groups of SHRs were removed rapidly for determination of renal norepinephrine content to verify the effectiveness of the serial renal denervation procedures. Both kidneys were

minced and placed in ice-cold 0.1 *N* perchloric acid. The kidneys were then homogenized, and the homogenate was centrifuged for 20 min at 20,000*g*. The supernatant was stored at −85°C until norepinephrine content was determined by high-pressure liquid chromotography with electrochemical detection (15).

Following sacrifice of the rats, the hearts were removed for determination of heart to body weight ratios in the three groups. The major blood vessels were transected at their sites of entry or exit from the heart. Prior to weighing the hearts, the ventricles were incised and the hearts were blotted on gauze to remove blood and surface moisture.

Statistical analysis. The tail-cuff pressures in the three groups of rats were compared by analysis of variance for repeated measures (16). When the analysis of variance indicated that overall group differences were present, the data during individual weeks were analyzed by the Bonferroni method for making multiple comparisons (17). Standard analysis of variance in conjunction with the Bonferroni method was utilized to compare MAP, body weights, and heart-to-body weight ratios among the three groups. The unpaired Student's *t* test was used for comparison of the renal norepinephrine content between the renal-denervated SHRs and the sham-operated SHRs. All values are reported as means ± SE, and $P < 0.05$ was accepted as a statistically significant difference.

Results. The initial tail-cuff pressures were measured prior to the first renal denervation or sham denervation procedures when the rats were 6 weeks old. At this time the arterial pressure averaged 95 ± 1.9 mm Hg (mean ±SE) in the 12 WKY rats, 113 ± 4.4 mm Hg in the 10 sham-implanted SHRs, and 112 ± 2.9 mm Hg in the 10 thymus-implanted SHRs. Therefore, both groups of SHRs had slightly higher tail-cuff pressures than did WKY control rats at this age. The 6-week-old WKY rats weighed slightly more (118 ± 3.1 g) than the sham-implanted (89 ± 3.1 g) or thymus-implanted SHRs (91 ± 1.9 g). Four of the SHRs in the implanted, denervated group died as a result of hemorrhage from the renal blood vessels at Week 9 during the first repetitive renal denervation procedure. A fifth rat in the implanted, denervated SHR group died inexplicably prior to the final tail-cuff pressure measurements of Week 20.

As shown in Fig. 1 the sham-implanted, sham-denervated SHRs gradually developed severe hypertension and the tail-cuff pressure reached a plateau level of approximately 195 mm Hg by the time these rats were about 4 months old. In sharp contrast, the tail-cuff pressure in thymus-implanted, renal-denervated SHRs increased slowly to a plateau level of only 140 mm Hg. The sham-denervated WKY rats maintained a tail-cuff pressure of approximately 120 mm Hg. The multiple comparison tests indicated that the tail-cuff pressures in both the WKY rats and the implanted, denervated SHRs were significantly less than those in the sham-operated SHRs during every measurement from Week 8 through Week 20. The tail-cuff pressures in the implanted, denervated SHRs were significantly higher than those in the WKY rats only at Weeks 11, 12, 14, 16, and 20.

The direct measurements of MAP in the conscious rats when they were 21 weeks old

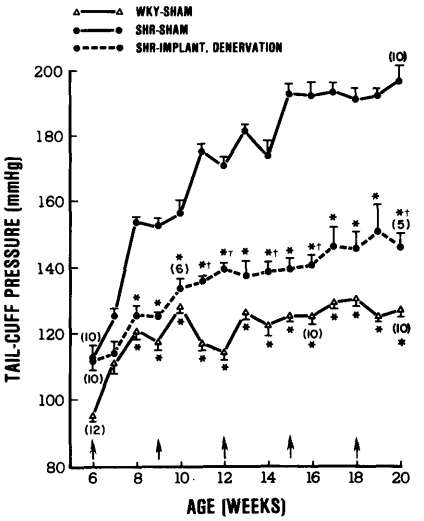

FIG. 1. Antihypertensive effect of thymic implants in combination with serial bilateral renal denervations in spontaneously hypertensive rats (SHRs). Implants of thymic tissue from donor Wistar rats were given to the SHRs neonatally and denervations were performed at arrows. Arterial pressures were also determined in sham-operated SHRs and sham-operated Wistar-Kyoto (WKY) rats. Numbers in parentheses are number of rats in the experiment as it progressed, and bars represent SE. **P* < 0.05 of WKY sham vs SHR sham or SHR implant, denervation vs SHR sham; †*P* < 0.05 of SHR implant, denervation vs WKY sham.

also indicated that the combination of neonatal thymic implants and serial renal denervations exerted a marked antihypertensive effect (Table I). If one considers the MAP of 121 mm Hg found in the WKY rats at this time to be normal, then these measurements show that approximately 77% of the spontaneous hypertension was prevented by the combined treatment. The measurements of heart-to-body weight ratio also indicated that the spontaneous hypertension was dramatically reduced in the implanted, denervated SHRs. Sham-operated SHRs had a heart-to-body weight ratio approximately 31% greater than that in the sham-operated WKY rats. The majority of this increase was prevented by the neonatal thymic implants in combination with renal denervation, so that the heart-to-body weight ratio in the treated SHRs was no longer significantly different than that in the WKY rats.

Measurements of renal norepinephrine content in the SHRs indicated that the serial renal denervations were effective (Table I). The renal norepinephrine content in the denervated SHRs was only approximately 17% of that found in the sham-operated SHRs 3 weeks after the fifth and final bilateral renal denervation.

Discussion. One important factor in the etiology of hypertension in the Okamoto spontaneously hypertensive rat is overactivity of the renal sympathetic nerves. Liard (4) demonstrated that bilateral renal denervation of young SHRs would delay development of hypertension in these rats by several weeks. This procedure did not alter the hypertension chronically. However, it was determined later that functional regeneration of the renal nerves in rats begins to occur in approximately 1 month (14). Therefore, the time course of possible reinnervation of the kidneys coincided with the period of delay in the development of the spontaneous hypertension. In order to determine whether increased renal nerve activity might be important in the chronic phase of hypertension in the SHR, we (5) prevented functional renal nerve regeneration by performing repetitive bilateral renal denervations at 3-week intervals in the aging rats. This chronic renal denervation resulted in both a delay in development of the hypertension and an amelioration of the spontaneous hypertension chronically. The renal-denervated SHRs became only about two-thirds as hypertensive as control SHRs. Other forms of interference with the activity of the sympathetic nervous system have been demonstrated to exert similar antihypertensive effects in the SHR (5, 18).

The chronic antihypertensive effect of renal denervation appears to be specific to the SHR in that removal of the renal nerves does not have a general effect to lower arterial pressure chronically. Successive renal denervations have no effect on arterial pressure in Wistar-Kyoto control rats (5). In addition, we have demonstrated that similar repetitive renal denervations have no effect on development or maintenance of DOC-salt hypertension (12) or on the chronic hypertensive level in

TABLE I. MEASUREMENTS IN 21-WEEK-OLD SHAM-OPERATED WISTAR-KYOTO (WKY) RATS, SHAM-OPERATED SPONTANEOUS HYPERTENSIVE RATS (SHRs), AND SHRs THAT RECEIVED THYMIC IMPLANTS NEONATALLY FROM DONOR WISTAR RATS AND, IN ADDITION, UNDERWENT REPETITIVE BILATERAL RENAL DENERVATION PROCEDURES

	WKY-Sham (n = 10)	SHR-Implant, Denervation (n = 5)	SHR-Sham (n = 10)
Mean arterial pressure (mm Hg)	121 ± 2.1*	134 ± 2.7*,**	177 ± 5.5
Body weight (g)	388 ± 12.8*	334 ± 10.8**	322 ± 7.4
Heart weight (g) per 100 g body weight	0.294 ± .006*	0.317 ± .016*	0.386 ± .007
Renal norepinephrine (ng/g kidney)	—	26.9 ± 3.7*	161 ± 16.8

Note. Values are means ± SE.
* $P < 0.05$ of WKY-Sham vs SHR-Sham or SHR-Implant, Denervation vs SHR-Sham.
** $P < .05$ of SHR-Implant, Denervation vs WKY-Sham.

rats with one-kidney, one-clip renal hypertension (13).

The concept that an abnormality of the immune system is important in the etiology of spontaneous hypertension is more recent. Bendich *et al.* (6) and Takeichi *et al.* (7) have demonstrated that the functions of T lymphocytes are suppressed in the SHR. This reduction in the activity of the T-lymphocyte system in the SHR is probably the result of gradual deterioration of thymic function in these rats (19). Notably, there is a reduction in the activity of T-suppressor cells in the SHR, and this type of change is frequently associated with autoimmune disorders. Manipulations of the immune system that decrease its overall activity (6, 8) or that reverse its dysfunction by increasing the activity of the T-suppressor lymphocytes toward the levels found in normotensive rats (9–11) attenuate the spontaneous hypertension. The effectiveness of these diverse types of immunologic treatments is compatible since suppression of the entire immune response would be expected to directly prevent any damaging effects of abnormal immune reactions, while reversal of the T-suppressor lymphocyte defect would correct the basic abnormality that leads to the immunologic dysfunction.

In a manner similar to the specificity of the effects of chronic renal denervation in the SHR, the effects of manipulations of the immune system are not generally antihypertensive. We have shown that chronic immunosuppressive treatment of normal Wistar or WKY rats has no effect on their arterial pressure (8). In addition, chronic immunosuppressive therapy has no effect on the development or maintenance of DOC-salt hypertension (8). We have also determined that implants of thymic tissue from donor SHRs have no effect on the arterial pressure of Wistar rats, and that thymic implants from donor Wistar rats have no effect on the development or maintenance of one-kidney, one-clip hypertension (11).

It remains to be determined exactly how the abnormality of the immune system in the SHR leads to development of hypertension. The immunologic defect in the SHR may raise the arterial pressure by causing vascular inflammation (20) or renal glomerular damage (21). There is increasing evidence that immunologic mechanisms may play a role in the etiology of arterial pressure increases in other models of hypertension as well as in some cases of essential hypertension in humans (22–24).

The results of this study indicate that the combination of neonatal thymic implants and chronic renal denervation delays development of hypertension in the SHR and attenuates the hypertension chronically. The average tail-cuff pressure of the implanted, denervated SHRs did not become significantly greater than that of the sham-operated WKY rats until these rats were about 3 months old, whereas the pressure in sham-operated SHRs was elevated compared with that in the WKY rats initially at 2 months of age. Following this time the arterial pressure of the sham-operated SHRs rose rapidly, while it increased slowly to only borderline hypertensive levels in implanted, denervated SHRs.

Thymic implants in combination with repetitive renal denervation chronically prevent approximately three-fourths of the spontaneous hypertension and the majority of the cardiac hypertrophy that normally occurs in the aging SHR. The degree of amelioration of the spontaneous hypertension suggests that the effects of reducing sympathetic activity and altering immune function do not interact in an antagonistic or synergistic fashion (25, 26). However, it is possible that these two factors which contribute importantly to the etiology of spontaneous hypertension may be linked. SHRs have been reported to be sensitive to the effects of stress, and this could result in both overactivity of the sympathetic nervous system and dysfunction of the immune system in these rats (27).

In conclusion, the results of this study indicate that the majority of the hypertension in the Okamoto SHR is the result of overactivity of the sympathetic nervous system and dysfunction of the immune system in these rats. The slightly higher arterial pressures of the implanted, denervated SHRs than those found in the control WKY rats might be due to one of several possible factors. The effects of the chronic renal denervations and of the neonatal thymic implants from the donor Wistar rats may not have been totally successful in reversing 100% of the effects of elevated sympathetic nervous activity or immunologic dysfunction, respectively, in the SHR. Elevated sympathetic nerve activity might also have a chronic effect on arterial pressure in

SHRs via an elevation in circulating catecholamines (28), and renal denervation would not have removed this mechanism. Finally, it is possible that an additional factor may play a role in the pathogenesis of spontaneous hypertension.

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1. Guyton AC, Coleman TG, Cowley AW Jr, Manning RD Jr, Norman RA Jr, Ferguson JD. A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. Circ Res **35**: 159–176, 1974.
2. Iriuchijima J. Sympathetic discharge rate in spontaneously hypertensive rats. Japan Heart J **14**:350–356, 1973.
3. Judy WV, Watanabe AM, Henry DP, Besch HR Jr, Murphy WR, Hockel GM. Sympathetic nerve activity: Role in regulation of blood pressure in the spontaneously hypertensive rat. Circ Res **38**(Suppl. II):21–29, 1976.
4. Liard JF. Renal denervation delays blood pressure increase in the spontaneously hypertensive rat. Experientia **33**:339–340, 1977.
5. Norman RA, Jr, Dzielak, DJ. Role of renal nerves in onset and maintenance of spontaneous hypertension. Amer J Physiol **243**:H284–H288, 1982.
6. Bendich A, Belisle EH, Strausser HR. Immune system modulation and its effect on the blood pressure in spontaneously hypertensive male and female rat. Biochem Biophys Res Commun **99**:600–607, 1981.
7. Takeichi N, Suzuki K, Kobayashi H. Characterization of immunological depression in spontaneously hypertensive rats. Eur J Immunol **11**:483–487, 1981.
8. Khraibi AA, Norman RA Jr, Dzielak DJ. Chronic immunosuppression attenuates hypertension in Okamoto spontaneously hypertensive rats. Amer J Physiol **247**(Heart Circ Physiol **16**):H722–H726, 1984.
9. Ba D, Takeichi N, Kodama T, Kobayashi H. Restoration of T cell depression and suppression of blood pressure in spontaneously hypertensive rats (SHR) by thymus grafts or thymus extracts. J Immunol **128**: 1211–1216, 1982.
10. Strausser HR. Immune response modulation in the spontaneously hypertensive rat. Thymus **5**:19–33, 1983.
11. Norman RA Jr, Dzielak DJ, Bost KL, Khraibi AA, Galloway PG. Immune system dysfunction contributes to the aetiology of spontaneous hypertension. J Hypertension **3**:261–268, 1985.
12. Dzielak DJ, Norman RA Jr. Renal denervation has no effect on the onset or maintenance of DOC-salt hypertension in rats. Amer J Physiol **249**(Heart Circ Physiol **18**):H945–H949, 1985.
13. Norman RA Jr, Murphy WR, Dzielak DJ, Khraibi AA, Carroll RG. Role of the renal nerves in one-kidney, one clip hypertension in rats. Hypertension **6**: 622–626, 1984.
14. Kline RL, Mercer PF. Functional reinnervation and development of supersensitivity to NE after renal denervation in rats. Amer J Physiol **238**(Renal Fluid Electrolyte Physiol 7):R353–R358, 1980.
15. Moyer TP, Jiang N-S. Optimized isocratic conditions for analysis of catecholamines by high-performance reversed-phase paired-ion chromatography with amperometric detection. J Chromatogr **153**:365–372, 1978.
16. Dixon WJ. BMDP Statistical Software. Berkeley, Calif., Univ. of California Press, 1981.
17. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. Circ Res **47**: 1–9, 1980.
18. Ciriello J, Kline RL, Zhang T-X, Caverson MM. Lesions of the paraventricular nucleus alter the development of spontaneous hypertension in the rat. Brain Res **310**:355–359, 1984.
19. Takeichi N, Ba D, Suzuki K, Kobayashi H. The arrest of T cell maturation in spontaneously hypertensive rats with a deficient production of thymic factors. Acta Pathol Japon **35**:351–360, 1985.
20. Suzuki T, Oboshi S, Sato R. Periarteritis nodosa in spontaneously hypertensive rats. Incidence and distribution. Acta Path Japon **29**:697–703, 1978.
21. Evan AP, Luft FC, Gattone V, Connors BA, McCarron DA, Willis LR. The glomerular filtration barrier in the spontaneously hypertensive rat. Hypertension **3**(Suppl. I):154–161, 1981.
22. Mathews JD, Whittingham S, Mackay IR. Autoimmune mechanisms in human vascular disease. Lancet **2**:1423–1427, 1974.
23. Svendsen UG. Immunological aspects of hypertension. Int J Immunopharmacol **1**:81–84, 1979.
24. Kristensen BO. Aspects of immunology and immunogenetics in human essential hypertension with special reference to vascular events. J Hypertension **2**: 571–579, 1984.
25. Ader R, Cohen N, Bovbjerg D. Conditioned suppression of humoral immunity in the rat. J Comp Physiol Psychol **96**:517–521, 1982.
26. Blalock JE. The immune system as a sensory organ. J Immunol **132**:1067–1070, 1984.
27. Keller SE, Weiss JM, Schleifer SJ, Miller NE, Stein M. Suppression of immunity by stress: effect of a graded series of stressors on lymphocyte stimulation in the rat. Science **213**:1397–1400, 1981.
28. Borkowski KR, Quinn P. Adrenaline and the development of genetic hypertension. J Hypertension **2**(Suppl 3):81–83, 1984.

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