Development of Left Ventricular Hypertrophy in Young Spontaneously Hypertensive Rats after Peripheral Sympathectomy

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SUMMARY The effects of peripheral sympathectomy with nerve growth factor antisemur (NGFAS) on blood pressure, systemic hemodynamics, myocardial function, myocardial hypertrophy, and renin were studied in male spontaneously hypertensive (SH) rats of the Okamoto strain and normotensive control Kyoto-Wistar (WKY) rats. NGFAS prevented the development of hypertension in the SH rats but did not alter blood pressure in the WKY rats. NGFAS treatment further depressed ventricular function in the SH rats, but had little effect on the WKY rats. Although NGFAS treatment effectively prevented the development of hypertension in the SH rats, it did not influence the development of left ventricular hypertrophy as reflected by increases in left ventricular mass, RNA, DNA, and hydroxyproline content. The data suggest that the development of myocardial hypertrophy and myocardial dysfunction in the SH rat is in part independent of hypertension and plasma renin activity.

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acceleration (dp/dt), were diminished in the SH rats compared to the WKY rats. NGFAS treatment further depressed ventricular function in the SH rats, but had little effect on the WKY rats. Plasma renin activity in both the SH and WKY rats was unaffected by NGFAS treatment. Although NGFAS treatment effectively prevented the development of hypertension in the SH rats, it did not influence the development of left ventricular hypertrophy as reflected by increases in left ventricular mass, RNA, DNA, and hydroxyproline content. The data suggest that the development of myocardial hypertrophy and myocardial dysfunction in the SH rat is in part independent of hypertension and plasma renin activity.
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The first derivative of pressure (dP/dt) and flow acceleration (dF/dt) were obtained with a resistance-capacitance differentiating circuit. Various indices of venous and myocardial hypertrophy have been shown previously to remain stable for at least 60 minutes. A catheter was inserted into the abdominal aorta in a group of SH rats. The rats were housed in air-conditioned quarters in group cages with no more than six rats per cage and fed Purina rat chow ad libitum. A 6 a.m. on-6 p.m. off environmental light cycle was maintained. Beginning when the rats were 1 day old and continuing daily until 7 days of age, NGFAS (Burroughs Wellcome) was injected subcutaneously in increasing doses of 0.05, 0.05, 0.1, 0.1, 0.2, 0.2, and 0.3 ml. Sham-treated SH and WKY rats received identical amounts of saline. Mortality following NGFAS treatment was 10% for both WKY and SH rats. Beginning at 40 days of age, the systolic blood pressure of the conscious rats was measured weekly using a modification of the tail cuff method of Pfeffer et al. Pressure tracings were recorded on a Lumiscrbe electrocardiograph (Monotronics). Five pressure measurements were recorded for each rat; the median of these readings was taken as the systolic blood pressure. Pulse rate was obtained from the pressure tracings. The tail cuff method was validated by comparison with blood pressures obtained through an indwelling catheter in the abdominal aorta in a group of SH rats.

At 80 days of age NGFAS- and saline-treated SH and WKY rats were anesthetized with sodium pentobarbital (Nembutal), 50 mg/kg, ip, and hemodynamic studies in situ were made. The rats were ventilated via a tracheostomy with a model 680 Harvard respirator at a rate of 40/min and a tidal volume of 2-3 ml. Arterial blood pH, P02, and PC02 have been shown previously to remain stable for at least 60 minutes. A catheter was inserted into the bifurcation of the abdominal aorta for measurement of aortic pressure with a Statham P23Db pressure transducer. The heart and great vessels were exposed through a midline sternotomy. Mean and phasic aortic flow were measured with a square wave electromagnetic flow probe around the ascending aorta and a model 400 flowmeter (Carolina Electronics). High fidelity left ventricular pressure was measured through a 3.8-cm, 22-gauge needle connected directly to a Statham P37 miniature pressure transducer. The frequency response of this system is linear within ±3.0 dB to 75 Hz. All recordings were made with a 16-channel photographic recorder (Electronics for Medicine). The first derivative of pressure (dP/dt) and flow acceleration (dF/dt) were obtained with a resistance-capacitance differentiating circuit. Various indices of ventricular function including peak flow velocity, stroke power, and stroke work were calculated from the left ventricular pressure and aortic flow tracings as previously described. Cardiac index was calculated from the mean aortic flow tracing. Instantaneous peak flow velocity was calculated by averaging the maximal value of at least 20 phasic flow curves. Stroke power was calculated from the integral of the product of the instantaneous flow and pressure curves. The factor 0.0143 was used to convert mm Hg ml/sec into g-m/sec. Stroke work per beat was obtained by dividing the stroke power by the heart rate.

Immediately after the hemodynamic study the hearts were excised, divided, and weighed. The interventricular septum was included with the left ventricle. The left ventricles were assayed for RNA, DNA, and hydroxyproline as previously described.

A second group of SH and WKY rats was killed at age 80 days by decapitation without anesthesia and exsanguinated. Blood was collected in iced tubes containing ethylenediaminetetraacetic acid (EDTA) (1 mg/ml) for measurement of renin activity, or heparin (147 U/tube) for measurement of dopamine β-hydroxylase activity. The brain minus olfactory bulbs was removed; dissected into telencephalon minus septum and corpus striatum, diencephalon, midbrain, and pons-medulla; and stored in liquid nitrogen for catecholamine analysis. The cerebrospinal cord, heart, and spleen were removed and stored in liquid nitrogen for subsequent norepinephrine analysis. The kidneys were removed and stored in liquid nitrogen for renin analysis.

Plasma renin activity was measured by radioimmunoassay of generated angiotensin I (AI). Plasma dopamine β-hydroxylase was measured by an enzymatic method.

Tissue catecholamines were determined by a modification of the method of Anton and Sayre, using reduced amounts of tissue, reagents, elution, and oxidation volumes.

Renal renin content was determined by a modification of the methods of Haas et al. and Boucher et al. Kidneys were thawed slowly at 4°C and refrozen three times over 3 successive days. Tissue was homogenized with a 7-ml hand grinder (Kimax) in a solution of 0.9% sodium chloride containing 1% EDTA, pH 4.9, (1 ml of solution/g of tissue) at 4°C. The hand grinder was rinsed with an equal volume of EDTA-saline solution and set aside. The tissue suspension was centrifuged at 35,000 g for 90 minutes in a refrigerated centrifuge (Sorvall), and the supernatant fraction was removed and stored at 4°C. The pellet was resuspended in the rinse solution from the grinder and centrifuged at 4°C for 30 minutes at 35,000 g. The supernatant fractions were combined and stored at 20°C.

For determination of renin content, the supernatant fractions were thawed at 4°C and a sample was diluted 1:100 with the 0.9% NaCl-1% EDTA solution. The diluted extract (50 ml) was added to 19.1 mg of partially purified rat substrate (enough to generate 6,000 ng of AI/ml) in 0.5 ml of 67 mm phosphate buffer, pH 7.4, containing 0.2% neomycin sulfate, 5 μl of quinolinol sulfate (6.8 mm final concentration) and 1 μl of dimercaprol (3.2 mm final concentration). The substrate had been prepared in our laboratory from the plasma of 24-hour nephrecto-
nmized rats by the method of Boucher et al.20 After removal of a zero time sample, the mixture was incubated for 15 minutes at 37°C. The reaction was stopped by freezing in a dry ice-acetone bath; samples of the reaction mixture were diluted 1:20 and 1:40 with phosphate buffer and subjected to radioimmunoassay for A 1.10 The zero time blank was assayed without dilution and subtracted from the generated value. Results were expressed as International units (IU) of renin per gram of kidney.21

Results

Systolic hypertension appeared in the SH rats at about 5–6 weeks of age and increased in severity with age (Fig. 1, top). Treatment with NGFAS prevented this increase. The systolic pressure of the awake, treated SH rats was not significantly different from the WKY controls (Fig. 1, bottom). Although the WKY rats exhibited an increase in blood pressure with age, there was no difference between the treated and sham-treated WKY rats. Heart rates in conscious rats at the time of death were the same in all four groups: treated SH rats 411 ± 9 (mean ± SEM) beats/min; control SH rats, 420 ± 5; treated WKY rats, 407 ± 11; control WKY rats, 411 ± 9.

The data from the hemodynamic study at 80 days of age are summarized in Figures 2 and 3. Heart rate was not significantly different among the four treatment groups. Left ventricular systolic pressure of the sham-treated SH rats was significantly higher than that of the NGFAS-treated SH rats and the treated and sham-treated WKY rats. These results were similar to the pressure data from the conscious rats although the pressures recorded in the anesthetized rats were uniformly lower in all groups. NGFAS effectively prevented the development of hypertension in the SH rats but did not affect blood pressure in the WKY rats. Left ventricular end-diastolic pressures were not significantly different among the four groups of rats.

The cardiac indices of the NGFAS- and sham-treated SH rats were significantly lower than those of both WKY treatment groups. The NGFAS treatment did not affect cardiac index in either the WKY or SH rats. Heart rates were comparable among the four treatment groups; therefore the changes in cardiac output reflect changes in stroke volume.

The calculated peripheral vascular resistance in the normotensive NGFAS-treated SH rats was significantly higher than that in the WKY controls but significantly lower than the sham-treated SH rats. The apparent persistence of elevated peripheral vascular resistance in the SH rats after NGFAS treatment may reflect the low cardiac output found in both treated and untreated SH rats, as well as a peripheral vascular abnormality.

When various indices of ventricular performance were examined, a difference in the response of the SH and WKY rats to NGFAS treatment was noted (Fig. 3). Whereas the WKY rats were little affected by NGFAS treatment, ventricular performance of the NGFAS-treated SH rats was markedly depressed. All of the indices except flow acceleration (dF/dt) were significantly lower in the SH rats after NGFAS treatment. Flow acceleration was slightly decreased (20%) in the NGFAS-treated SH rats (0.05 > P < 0.1). Peak flow velocity and dF/dt were significantly depressed in the sham-treated SH rats compared to the sham-treated WKY rats.

The development of left ventricular hypertrophy in the SH rats was not affected by NGFAS treatment (Fig. 4). The left ventricular-body weight ratio, left ventricular RNA, DNA, and collagen (expressed as hydroxyproline) were all significantly greater in the NGFAS-treated SH rats than in the NGFAS- and sham-treated WKY rats. The mean absolute left ventricular weight of the treated SH
rats was significantly greater than that of the treated WKY rats. 562 ± 20 (mean ± SEM) mg compared to 502 ± 10 mg (P < 0.02), but not significantly different from that of the sham-treated SH rats, 588 ± 10 mg (not significant). Although NGFAS treatment prevented the development of measurable hypertension in the SH rats, it did not prevent the development of left ventricular hypertrophy.

NGFAS treatment did not affect plasma renin activity (Table 1). Kidney renin content was elevated in the NGFAS-treated SH rats; in contrast, NGFAS treatment did not affect kidney renin content in the WKY rats. Dopamine \( \beta \)-hydroxylase activity was lower in the NGFAS-treated SH rats than in the sham-treated rats.

NGFAS treatment did not affect regional brain norepinephrine concentrations in either the SH or WKY rats (Table 2). Spinal cord norepinephrine was similarly unaffected by NGFAS treatment in the SH rats. In contrast, NGFAS treatment caused profound depletions of myocardial and splenic norepinephrine, compatible with nearly complete sympathetic denervation of these organs.

Discussion

We have demonstrated that NGFAS administered to the newborn SH rat can inhibit the development of the peripheral sympathetic nervous system and prevent the appearance of the hypertensive syndrome. Antibodies to nerve growth factor inhibit RNA and protein synthesis in actively growing peripheral sympathetic nerve tissue. This results in permanent destruction of 95-98% of the paravertebral ganglia. The central nervous system, however, is unaffected by NGFAS treatment. In our study, NGFAS resulted in marked depletion of myocardial and splenic norepinephrine stores in both SH and WKY rats, compatible with nearly complete sympathetic denervation of these organs. In addition, plasma dopamine \( \beta \)-hydroxylase activity was decreased after NGFAS treatment in the SH rats suggesting decreased peripheral sympathetic activity. In contrast, central nervous system norepinephrine content was unaffected by NGFAS treatment in either the SH or WKY rats. These observations are consistent with the lack of effect of NGFAS on developing noradrenergic structures in the central nervous system and suggest that the hemodynamic and humoral changes seen after NGFAS treatment in the rat are related to peripheral sympathetic mechanisms.

NGFAS treatment did not affect the blood pressure of normotensive WKY rats despite the fact that the depletions of myocardial and splenic norepinephrine in the SH and WKY strains were similar. The lack of effect of NGFAS treatment on blood pressure in normotensive rats indicates that peripheral immunosympathectomy does not lead to a nonspecific lowering of blood pressure but, rather, interferes selectively with the development of hypertension in SH rats. In the present study peripheral sympathetic denervation with NGFAS prevented the development of hypertension but did not alter cardiac output. These data, therefore, do not support the concept that increased sympathetic activity alone is the origin of the hemodynamic abnormalities found in the SH rat.

Our studies and those of others have demonstrated abnormalities in myocardial function in SH rats when compared with normotensive controls. Peak flow velocity, maximum flow acceleration, and rate of pressure development, all indices of myocardial contractility, decreased in the hypertensive SH rat. Although Pfeffer and Frohlich found these abnormalities only in SH rats 24 weeks of age or older, we demonstrated depressed myocardial performance early in the development of the hypertensive syndrome. Since our study was completed at least 2 years after that of Pfeffer and Frohlich, a discrepancy may be related to the tendency of successive generations of the SH rat to develop hypertension at an earlier age. It is reasonable to expect that other manifestations of the hypertensive syndrome, such as myocardial dysfunction, will also become apparent in the younger animal.

Our findings suggest that alterations in ventricular performance in the SH rat are not entirely a result of hyper-

![Figure 3](http://circres.ahajournals.org/)

**Figure 3** Peak flow velocity (pFl Vel), stroke power (SP), stroke work (SW), maximum rate of pressure rise (dP/dt max) and flow acceleration (dF/dt), of the NGFAS- and sham-treated WKY and SH rats (abbreviations as in Figure 2). Values are expressed as the mean ± SEM. For each group n = 8.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** Body weight (BW), left ventricular-body weight ratio (LV/BW), left ventricular RNA, DNA, and hydroxyproline (OHPro) of NGFAS- and sham-treated WKY and SH rats (abbreviations as in Figure 2). Values are expressed as the mean ± SEM. For each group n = 8.
tension of long duration, but rather are related to some underlying myocardial abnormality. Treatment with NGFAS further depressed ventricular performance in the SH rats but had little effect on the WKY controls. Increased sympathetic activity may, therefore, play a compensatory role in the maintenance of myocardial function in the SH rat, as is apparent from the further decrease in ventricular performance following immunosympathectomy. It should be noted, however, that none of the WKY or SH rats demonstrated signs of congestive heart failure following immunosympathectomy, as evidenced by normal left ventricular end-diastolic pressure and absence of pleural effusion or hepatosplenomegaly.

Our data clearly demonstrate that left ventricular hypertrophy, measured as changes in left ventricular mass, RNA, DNA, and hydroxyproline, occurs in the spontaneously hypertensive rat in the absence of measurable systemic hypertension. At an age when sham-treated SH rats were significantly hypertensive compared to WKY rats, the NGFAS-treated SH rats remained normotensive but developed myocardial hypertrophy. An extension of this study to include rats 24 weeks of age has also demonstrated that NGFAS treatment prevents the development of hypertension but not hypertrophy (manuscript in preparation). These data suggest a dissociation between the presence of hypertension and the development of myocardial hypertrophy in the rat genetically predisposed to hypertension.

This conclusion is supported by the finding of increased left ventricular mass in the SH rat in the prehypertensive phase.4,11,27 Pfeffer et al.28 have reported that myocardial hypertrophy occurred in 10% of their normotensive WKY rats. Although we did not find evidence of myocardial hypertrophy in any WKY rat in this study, we regard their finding as further evidence that hypertrophy is not completely dependent on the occurrence of hypertension in the SH rat. Furthermore, it has recently been shown that electrocardiographic evidence for left ventricular hypertrophy in hypertensive patients does not correlate well with the degree of pressure elevation.29

The renin-angiotensin system has been implicated as an independent cause of myocardial hypertrophy in the SH rat. Sen et al.30 treated SH rats with hydralazine or α-methyldopa in doses that had equivalent antihypertensive efficacy. The hydralazine-treated rats developed left ventricular hypertrophy and elevated plasma renin activity but did not become hypertensive. The rats treated with α-methyldopa did not develop left ventricular hypertrophy, but plasma renin activity was depressed. Since angiotensin II has been shown to stimulate myocardial DNA, RNA, and protein synthesis,29 the high circulating renin in the hydralazine-treated rats was thought to play a permissive role in the development of myocardial hypertrophy.

### Table 1: Effect of Nerve Growth Factor Antiserum (NGFAS) Administration to Spontaneously Hypertensive (SH) and Normotensive Kyoto-Wistar (WKY) Rats on Renin and Dopamine β-Hydroxylase (DBH)

<table>
<thead>
<tr>
<th></th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Plasma renin activity (ng A/l/ml per hr)</th>
<th>Kidney renin content (IU/g)</th>
<th>Plasma DBH activity (μmol/liter per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH rats</td>
<td></td>
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</tr>
<tr>
<td>Sham (n = 10)</td>
<td>202 ± 12</td>
<td>1.6 ± 0.9</td>
<td>2.41 ± 0.17</td>
<td>0.86 ± 0.04</td>
</tr>
<tr>
<td>NGFAS (n = 8)</td>
<td>157 ± 3</td>
<td>1.5 ± 0.4</td>
<td>4.24 ± 0.31</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>WKY rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n = 8)</td>
<td>155 ± 3</td>
<td>2.3 ± 0.3</td>
<td>2.95 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>NGFAS (n = 6)</td>
<td>152 ± 7</td>
<td>3.4 ± 2.2</td>
<td>2.89 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</table>

Results are expressed as mean ± SEM; n = number of rats; NS = not significant.

### Table 2: Effect of Nerve Growth Factor Antiserum (NGFAS) Administration to Spontaneously Hypertensive (SH) and Normotensive Kyoto-Wistar (WKY) Rats on Tissue Catecholamine Levels

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (μg/g)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Telencephalon</td>
</tr>
<tr>
<td>SH rats</td>
<td></td>
</tr>
<tr>
<td>Sham (n = 8)</td>
<td>0.245 ± 0.008</td>
</tr>
<tr>
<td>NGFAS (n = 8)</td>
<td>0.237 ± 0.018</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
</tr>
<tr>
<td>WKY rats</td>
<td></td>
</tr>
<tr>
<td>Sham (n = 9)</td>
<td>0.246 ± 0.011</td>
</tr>
<tr>
<td>NGFAS (n = 9)</td>
<td>0.237 ± 0.016</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; n = number of rats; NS = not significant.
role in the development of myocardial hypertrophy. In our study plasma renin activity, measured in rats decapitated without prior anesthesia and exsanguinated, was the same in NGFAS-treated and sham-treated SH rats. These data do not support the concept that alterations in circulating renin account for the development of myocardial hypertrophy in the normotensive NGFAS-treated SH rat. Although at first it may appear that antihypertensive therapy with a-methyldopa prevents the development of hypertrophy, a closer look at the data indicates that a-methyldopa may have a nonspecific effect on myocardial cell growth. The left ventricular-body weight ratio in both the SH and control rats decreased after treatment. In a more recent paper20 this is also demonstrated by changes in RNA concentration as well as cardiac mass. The hearts of the treated SH rats remained hypertrophic as compared to treated control rats.

Another mechanism which may explain the early development of hypertrophy in the SH rat is the existence of a hyperdynamic cardiovascular system in the early stage of the syndrome. Although a high cardiac output state mainly due to an increase in heart rate has been reported in the SH rat, other studies21, 22 have failed to confirm this finding. In our study the heart rates of both the unanesthetized NGFAS- and sham-treated SH rats were similar to those of the unanesthetized WKY rats.

Kidney renin content was increased in the NGFAS-treated SH rats in the presence of normal plasma renin activity. The failure of plasma renin activity to increase in the NGFAS-treated SH rats may reflect the absence of an appropriate compensatory release of renin in response to blood pressure lowering. The fall in blood pressure may have triggered an increase in renin synthesis, but the absence of functioning renal sympathetic nerves prevented an increase in renin release. There is evidence that sympatholytic treatment affects both renin synthesis and the mobilization of renin stores from the rat kidney.23 Reserpine treatment was shown to produce acute increases in renal renin content and juxtaglomerular cell granulation and decreases in plasma renin activity in the Sprague-Dawley rat. Ultrastructural examination of the kidneys showed an increase in crystalline protogranules and Golgi vesicles, indicating increased cellular activity and suggesting enhanced renin synthesis immediately after reserpine treatment. In contrast, NGFAS treatment did not alter either renal renin content or plasma renin activity in the normotensive WKY rats. Since NGFAS did not produce changes in blood pressure in the WKY rats, the stimulus for altering renin synthesis or release, or both, may have been lacking.

To explain the development of left ventricular hypertrophy in the normotensive NGFAS-treated SH rat, we propose that myocardial hypertrophy may develop as a result of a genetic cardiovascular abnormality that does not require increased systemic pressure for its expression. In our hypothesis a primary myocardial abnormality in an animal genetically predisposed to hypertension results in myocardial dysfunction and hypertrophy. This is followed by a compensatory increase in sympathetic activity to maintain cardiac output; myocardial function returns toward, but not completely to, normal. Because of increased sympathetic activity, vasoconstriction occurs which results in increased peripheral vascular resistance and elevated systemic pressure. Other genetically determined factors, such as increased reactivity of the arteriole musculature24 or increased levels of circulating catecholamines,25 may also play a role. Because of high peripheral vascular resistance and continued hypertension, cardiac output is further diminished and left ventricular hypertrophy increases. In the above scheme if increased sympathetic activity is prevented by NGFAS, myocardial hypertrophy will still develop; even though pressure remains normal, ventricular function and cardiac output are still compromised.

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