


The pellet was resuspended in the rinse solution from the final concentration) and I of dimercaprol (3.2 mM of dimercaprol sodium disulfide) and I of quinolinol sulfate (6.8 mg/ml) in 0.2% neomycin sulfate, 5 ml 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 Lumiscribe 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.13 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 dosages 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 Lumiscribe 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.

Methods

Male SH rats and normotensive Kyoto-Wistar (WKY) rats (Taconic Farms) were used. 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 dosages 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 Lumiscribe 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.
Tension in the SH rats but did not affect blood pressure in
NGFAS effectively prevented the development of hyper-
esthesia of rats were uniformly lower in all groups.
These results were similar to the pressure data from
significantly different among the four treatment groups.
Heart rate was not significantly different from the WKY controls (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), control SH rats, 420 ± 5; control WKY rats, 411 ± 9; control 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

**Figure 1** Top: systolic blood pressures of unanesthetized nerve growth factor antiserum (NGF) grown rats (○) and sham-treated (○ ‑ ‑ ‑ ○) spontaneously hypertensive (SH) rats. Values are expressed as mean ± SEM. For each group, n = 16, P < 0.005. Bottom: systolic blood pressures of NGFAS-treated (○) and sham-treated (○ ‑ ‑ ‑ ○) Kyoto-Wistar (WKY) rats. Values are expressed as the mean ± SEM. For each group n = 16, P = NS.

**Figure 2** Heart rate (HR), peak left ventricular systolic pressure (SystBP), cardiac index (CI), and peripheral vascular resistance (PVR) of the nerve growth factor antiserum (NGFAS)-treated and sham-treated Kyoto-Wistar (WKY) and spontaneously hypertensive (SH) rats. Values are expressed as the mean ± SEM. For each group n = 8. *Units are mm Hg/ml per min.
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 β-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 β-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 studies4, 7 and those of others6, 24 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,25 are decreased in the hypertensive SH rat. Although Pfeffer and Frohlich6 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,6 this discrepancy may be related to the tendency of successive generations of the SH rat to develop hypertension at an earlier age.26 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-
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 1/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><strong>SH rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</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><em>P</em></td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>WKY rats</strong></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><em>P</em></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

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

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. Pfeffer et al. 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.

The renin-angiotensin system has been implicated as an independent cause of myocardial hypertrophy in the SH rat. Sen et al. 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, the high circulating renin in the hydralazine-treated rats was thought to play a permissive role in the development of myocardial hypertrophy.

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>
</thead>
<tbody>
<tr>
<td></td>
<td>Telencephalon</td>
</tr>
<tr>
<td><strong>SH rats</strong></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><em>P</em></td>
<td>NS</td>
</tr>
<tr>
<td><strong>WKY rats</strong></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><em>P</em></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 α-methyldopa prevents the development of hypertrophy, a closer look at the data indicates that α-methylldopa 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 paper 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 studies 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. 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 protoagranules 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 arteriolar musculature or increased levels of circulating catecholamines, 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.

References

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Development of left ventricular hypertrophy in young spontaneously hypertensive rats after peripheral sympathectomy.

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