Effects of Immunosympathectomy on Development of High Blood Pressure in Genetically Hypertensive Rats

By David W. J. Clark

ABSTRACT

Newborn male rats from the New Zealand colony with genetic hypertension (hypertensive rats) and from a normotensive colony (controls) were immunosympathectomized by injections of antiserum to nerve growth factor. Blood pressure of immunosympathectomized hypertensive rats aged 63 days (128 ± 2 mm Hg) was significantly less (P < .001) than that of untreated hypertensive rats (176 ± 3 mm Hg) of the same age. Pressure of immunosympathectomized controls aged 63 days (107 ± 2 mm Hg) was significantly less (P < .001) than that of untreated controls (126 ± 1 mm Hg) at the same age. Pressure thereafter remained near these levels. In immunosympathectomized rats, ganglion blockade with supramaximal doses of hexamethonium still induced a large fall in pressure which was significantly lower than in untreated rats. The combination of immunosympathectomy and hexamethonium brought the pressure of hypertensive rats and controls down to the same level (68 mm Hg). The pressor response to intravenous norepinephrine was greater in immunosympathectomized than in untreated rats. Resistance to flow in perfused mesenteric arteries from immunosympathectomized hypertensive rats was significantly less (P < .05) than in arteries from untreated hypertensive rats. Hearts of immunosympathectomized hypertensive rats were significantly lighter (P < .01) than those of untreated hypertensive rats. Heart rates of hypertensive or control rats were not altered by immunosympathectomy.

KEY WORDS
cardiac hypertrophy ganglion blockade norepinephrine isolated blood vessels sympathetic stimulation

The sympathetic nervous system plays an important part in maintaining the blood pressure and peripheral resistance at abnormally high levels in mature rats from the New Zealand strain with genetic hypertension (hereafter called hypertensive rats) (1-3). The mechanisms by which this is achieved have yet to be fully clarified but may involve increased in vivo sympathetic nervous activity at sympathetic endings, enhanced reactivity of smooth muscle to nervous stimulation, or any combination of these factors. The blood pressure of these rats is significantly above that of a random-bred control strain at 2 days of age and increases until the age of 70 days (4). Cardiac hypertrophy is present in the hypertensive rats from 45 days of age (5), and at maturity they also show increased vascular reactivity to norepinephrine (NE) and other agents (6-8).

Treatment of newborn rats with antiserum to nerve growth factor (9, 10) prevents the development of much of their sympathetic nervous system. Hypertensive and control rats were treated with antiserum (immunosympathectomized) to examine the role of the sympathetic nervous system in the production of hypertension in the genetically hypertensive rats. Some of these findings have already been reported in abstract form (11, 12).
Materials and Methods

Newborn male hypertensive and control rats were injected intraperitoneally daily for 5 days with antiserum to nerve growth factor (Wellcome Reagents Ltd., batch Ex 3012/12/14). About half the male rats in all litters of rats used for this series were not injected and served as controls. Rats were weaned at 28 days. Systolic blood pressure was measured under light ether anesthesia by a tail cuff method (13) at 10, 21, 42, 63, and 84 days and after 200 days. Heart rate was determined in rats aged 84 days by electronically counting the QRS complexes of the ECG over 30-second periods. The mean of three consecutive counts, which usually differed by less than 5%, was taken as the heart rate.

Intraarterial blood pressure was measured (under chloralose anesthesia, 75 mg/kg) from the cannulated femoral arteries of weight- and age-matched hypertensive rats (30 pairs of immunosympathectomized and untreated rats) and of weight- and age-matched control rats (29 pairs). Measurements were made with small-volume mercury manometers and recorded kymographically. When the blood pressure was stable, the responses to injections into the femoral vein of hexamethonium bromide (20 mg/kg) and to norepinephrine (NE, as Levophed, 0.5 μg base/kg), both before and after hexamethonium, were measured.

At the end of these experiments the rats were killed, while still under anesthesia, by intravenous air injections. The hearts were removed, cleaned, blotted dry and weighed. Both superior cervical ganglia and, in some of the rats, the nerve plexus containing the celiac-mesenteric ganglionic complex, were excised, fixed in 10% buffered formalin, and embedded in paraffin. Serial longitudinal sections were stained with haematoxylin and eosin or with van Gieson's stain. Nerve cells were identified by the presence of a large ovoid nucleus and cytoplasm of a granular appearance. Usually two or more nucleoli were visible in these cells. Nerve cells of sections stained with haematoxylin-eosin from the midarea of superior cervical ganglia were counted with the aid of a grid in the eyepiece of a light microscope. The largest number of nerve cells from either the left or right superior cervical ganglia from each rat was taken as the midsection number. Van Gieson-stained sections were examined for the presence of collagen.

Mesenteric vascular beds from pairs of rats aged 63 days and over were isolated and prepared for perfusion by a technique modified from that described by McGregor (14). Only one main branch of the anterior mesenteric artery, instead of four, was perfused with Locke's physiological saline solution (15) at a constant rate of 2 ml/min. NE (0.5 μg) in Locke's solution was injected through the arterial cannula into the perfusate at 2-minute intervals until similar responses were obtained on three consecutive occasions. The periarterial sympathetic plexus was stimulated with bursts of 100 pulses (75 v, 16 Hz, 5-msec duration) at 2-minute intervals until consistent responses were obtained. Perfusion pressure (directly proportional to vascular resistance) was measured with a small-volume mercury manometer connected by a T-piece to the output of the perfusion pump.

Results were analyzed with Student's t-test and, unless otherwise stated, are given as means ±SE.

Results

The weight and general health of immunosympathectomized rats was similar to that of untreated rats. Ptosis was present in immunosympathectomized rats from an early age.

Sympathetic Ganglia

Macroscopically, the superior cervical ganglia of immunosympathectomized rats were about one-third of the size of those of untreated rats. The mean number of nerve cells in midsections of superior cervical ganglia was 730 ± 64 in 12 untreated rats, and 109 ± 11 in 35 immunosympathectomized rats. The amount of collagen present in ganglia from immunosympathectomized rats was greater than that in ganglia from untreated rats. In the celiac-mesenteric ganglionic complexes of four immunosympathectomized and four untreated rats, no differences in nerve cell density could be demonstrated. These ganglia were too irregular in shape for comparisons of nerve cell numbers.

Blood Pressure

By the age of 81 days, the blood pressure (tail cuff) of immunosympathectomized rats from both colonies was significantly lower (P < 0.001) than that of untreated rats (Fig. 1). The blood pressure differences were still present in rats aged over 200 days. The effect of immunosympathectomy was confirmed by intraarterial recordings in matched pairs of immunosympathectomized and untreated rats aged 63 to 70 days, and the blood pressure of the immunosympathectomized hypertensive rats was 758 ± 2% of the untreated value.
FIGURE 1

Rise of blood pressure with age in genetically hypertensive rats (GH) and control rats (N). At ages 10 to 63 days readings were obtained from 25 to 60 animals; at 84 days, from 17 rats in each group; and after 200 days, from 6 rats from each group three times at weekly intervals. ia = intraarterial.

While that of the immunosympathectomized control rats was 908 ± 25 of the untreated value.

Heart rates and heart weights

Immunosympathectomy did not change heart rate significantly. In 17 immunosympathectomized hypertensive rats aged 84 days, the heart rate was 428 ± 5 beats/min compared with 433 ± 5 beats/min in 17 untreated hypertensive rats of the same age. In 17 immunosympathectomized control rats aged 84 days, the mean heart rate was 391 ± 4 beats/min compared with 394 ± 6 beats/min in 17 untreated control rats.

In 28 immunosympathectomized hypertensive rats aged 63 to 70 days, the mean heart weight was 630 ± 25 mg (344 ± 7 mg/100 g body weight), while in 28 age-matched untreated hypertensive rats, the mean heart weight was significantly greater (P < 0.01), the comparable value being 734 ± 30 mg (400 ± 9.5 mg/100 g body weight). The mean heart weight of 27 immunosympathectomized control rats of the same age was 737 ± 31 mg (323 ± 6 mg/100 g body weight); this was not significantly different from the heart weight in matched untreated control rats, which was 835 ± 36 mg (329 ± 5 mg/100 g body weight).

Cardiovascular responses in the whole animal (Table 1)

Following ganglion blockade with hexamethonium, the blood pressure fell both in immunosympathectomized and in untreated rats. In the hypertensive rats, the fall was greater in the untreated than in the immunosympathectomized rats (P < 0.05). In the control rats also, the fall was greater in the untreated than in the immunosympathectomized rats, but the difference was not significant. After hexamethonium, the residual or floor blood pressure was significantly lower (P < 0.001) in the immunosympathectomized hypertensive rats than in the untreated hypertensive rats. Similarly, in the control rats after hexamethonium the residual blood pressure was lower (P < 0.05) in the immunosympathectomized than in the untreated rats. The combination of immunosympathectomy and hexamethonium brought the blood pressure of hypertensive and control rats down to the same level (68 mm Hg).

The pressor responses to NE were greater in immunosympathectomized rats from both colonies than they were in the corresponding untreated rats. Untreated hypertensive rats did not differ significantly from untreated control rats in their response to NE, nor did immunosympathectomized hypertensive rats differ in this respect from immunosympathectomized control rats. After ganglion blockade, the responses of hypertensive rats were only slightly greater than those of the correspond-
TABLE 1

<table>
<thead>
<tr>
<th>Hypertensive rats</th>
<th>Control rats</th>
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<td>Treated*</td>
<td>Untreated</td>
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<td>Treated*</td>
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Effect of Immunosympathectomy on Responses to Norepinephrine (0.5 μg/kg) and Hexamethonium (80 mg/kg)

**Initial BP**
- Hypertensive rats: 143 ± 2
- Treated*: 130 ± 3
- Untreated: 135 ± 3

**Pressor response to NE**
- Hypertensive rats: 65 ± 2
- Treated*: 42 ± 2
- Untreated: 68 ± 2

**BP**
- Hypertensive rats: 136 ± 3
- Treated*: 110 ± 2
- Untreated: 130 ± 3

**Fall in BP**
- Hypertensive rats: 70 ± 2
- Treated*: 51 ± 3
- Untreated: 56 ± 2

**Residual BP**
- Hypertensive rats: 95 ± 4
- Treated*: 89 ± 5
- Untreated: 74 ± 2

**Values are mean mm Hg ± SE for 24 to 27 pairs of immunosympathectomized and untreated rats 63 to 70 days old.**

*Immunosympathectomy. Significance of difference between treated and untreated, Student's *t*-test (unpaired): fP < 0.001; %P < 0.05.

With a constant perfusion rate, the pressure developed in unstimulated mesenteric arteries isolated from immunosympathectomized hypertensive rats was significantly less (P<0.05) than in arteries from untreated hypertensive rats. There was no difference in the perfusion pressure developed in preparations from immunosympathectomized and untreated control rats. When untreated hypertensive rats were compared with untreated control rats, the perfusion pressure in preparations from the hypertensive rats was significantly higher (P<0.001). However, when preparations from immunosympathectomized hypertensive rats were compared with preparations from immunosympathectomized control rats, there was no difference in perfusion pressure.

The response to periarterial sympathetic nerve stimulation in preparations from immunosympathectomized rats of both colonies was significantly less (both P<0.001) than

**TABLE 2**

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<th>Perfusion pressure</th>
<th>Hypertensive rats (22)</th>
<th>Control rats (22)</th>
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<td></td>
<td>Treated*</td>
<td>Untreated</td>
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- Baseline (flow rate 2 ml/min)
  - Hypertensive rats: 36 ± 8
  - Treated*: 32 ± 8
  - Untreated: 37 ± 8

- Response to NE
  - Hypertensive rats: 118 ± 11
  - Treated*: 115 ± 10
  - Untreated: 110 ± 10

- Response to sympathetic stimulation
  - Hypertensive rats: +21 ± 32
  - Treated*: +63 ± 7
  - Untreated: +14 ± 32

**Values are mean mm Hg ± SE for 24 to 27 pairs of immunosympathectomized and untreated rats 63 to 70 days old.**

*Immunosympathectomized. Significance of difference between treated and untreated, Student's *t*-test (unpaired): fP < 0.05; %P < 0.001.
that in preparations from untreated rats. The vasoconstrictor response to injected NE was greater in preparations from immunosympathectomized control rats than in those from untreated control rats \( (P < 0.05) \). There was no difference in the response to NE of immunosympathectomized and untreated hypertensive rats.

**Discussion**

The degree of sympathectomy produced by treatment with antiserum to nerve growth factor, although considerable, was incomplete. This is consistent with results obtained by others on the effects of immunosympathectomy \( (10, 16, 17) \). The following findings indicate that a degree of sympathectomy was achieved. Rats treated with the antiserum exhibited ptosis from an early age. The number of nerve cells was greatly reduced in the superior cervical ganglia of immunosympathectomized rats. The response of the blood pressure to NE was increased in immunosympathectomized rats, as is typical of surgically or chemically sympathectomized animals. Mesenteric arteries from immunosympathectomized rats, although still showing some response, responded less to sympathetic stimulation than did preparations from untreated rats. The NE contents of, and the uptake of \(^{3}H\)-NE into, the hearts, spleens and submaxillary salivary glands of immunosympathectomized rats were greatly reduced, but the NE content and uptake of \(^{3}H\)-NE in the pancreas of these immunosympathectomized rats did not differ greatly from normal (Phelan, personal communication). These observations are consistent with those of Iversen et al. \( (16) \) and Zaimis et al. \( (17) \). Histological differences between immunosympathectomized and untreated rats could not be demonstrated in the celiac-mesenteric ganglionic complexes. However, it has been reported \( (18) \) that this ganglionic complex and other prevertebral ganglia are not affected as much by immunosympathectomy as are ganglia of the paravertebral chains. This selectivity of immunosympathectomy probably accounts for the differences between the effects of NE content and uptake in different tissues, and for the persistence of a response to sympathetic nervous stimulation in perfused mesenteric arteries from immunosympathectomized rats.

After immunosympathectomy, the blood pressure was lower and the response to NE was greater than in untreated rats. Following ganglion blockade, however, there was a further lowering of blood pressure and a further increase in the size of the response to NE, suggesting that the sympathectomy was not complete.

Immunosympathectomy prevented the blood pressure of both hypertensive and control rats from reaching the levels normally achieved in mature animals. Some other types of experimental hypertension also are affected in the same way by immunosympathectomy. For instance, the blood pressure of immunosympathectomized rats fed a diet containing triiodothyronine and excess NaCl did not rise to the high level reached by untreated rats on the same diet \( (19) \). Renal hypertension was initiated in immunosympathectomized rats, but a high blood pressure was not maintained \( (20) \). On the other hand, immunosympathectomy did not affect the production of metacorticoid and NaCl hypertension \( (21) \). In all types of experimental hypertension, apart from genetic hypertension, immunosympathectomy precedes the maneuver which causes the hypertension.

When the blood pressure (at 63 to 70 days) of immunosympathectomized rats was expressed as a percent of the pressure of the appropriate untreated rats, the effect of immunosympathectomy appeared greater in hypertensive rats than in control rats. Rats from the genetically hypertensive colony whose heart and blood vessels were not exposed to the high levels of blood pressure usual in this colony did not develop cardiac hypertrophy. The resistance to flow in the mesenteric arteries of immunosympathectomized hypertensive rats was significantly less than in the arteries from immunosympathectomized or untreated control rats.

The blood pressure of immunosympathectomized hypertensive rats remained higher.
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than that of immunosympathectomized control rats. It is possible either that the sympathetic system is less affected by immunosympathectomy in hypertensive rats or that the remaining sympathetic system exerts a greater effect due to a greater sensitivity of the effector organs to the neurotransmitter released by sympathetic stimulation. Alternatively, mechanisms of non-neurogenic origin may have been elevating the blood pressure of immunosympathectomized hypertensive rats. This, however, is unlikely because, while ganglion blockade of untreated rats still leaves a difference of blood pressure between hypertensive and control rats, no such difference persisted between ganglion-blocked immunosympathectomized hypertensive and control rats.

The fall in blood pressure in immunosympathectomized rats was large after hexamethonium was given. In these circumstances, ganglion blockade would lower blood pressure by affecting either the remaining sympathetic nerves to the blood vessels and heart or by the release of adrenal catecholamines. An increased turnover of adrenal catecholamines has been demonstrated in immunosympathectomized rats (16). This may reflect a mechanism which compensates for the reduced activity of other parts of the sympathetic system. Evidence of this has been provided by Brody, who reported that the resistance of the blood-perfused hindquarters of immunosympathectomized rats with their adrenal medullae removed is lower than that of hindquarters from immunosympathectomized rats with intact adrenals (22).

The elevated heart rate of hypertensive rats was not affected by immunosympathectomy and may reflect a genetic difference from control rats. This elevated heart rate remains a factor that may maintain the blood pressure of immunosympathectomized hypertensive rats at a higher level than is found in immunosympathectomized control rats. While the cardiac hyperventilation and the increased resistance of the mesenteric arteries of hypertensive rats could be secondary to the hypertension, it appears that the increased heart rate is not necessarily accompanied by blood pressure elevation.

It is concluded that the sympathetic nervous system plays a considerable role in the development of the high blood pressure, the cardiac hypertrophy, and the increased resistance to flow in mesenteric arteries of rats from the New Zealand colony with genetic hypertension.

Acknowledgment
I am grateful to Sir Horace Smith for suggesting the research, to Assoc. Prof. F. O. Simpson and to Mr. E. L. Phelan for advice and criticism and to Mr. M. Birtles for the histology.

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doi: 10.1161/01.RES.28.3.330

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