Vasopressor Hyperresponsiveness in New Zealand Genetically Hypertensive Rats

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SUMMARY The relationship of blood pressure (BP) to vascular hyperresponsiveness to norepinephrine (NE) in New Zealand genetically hypertensive (NZGH) rats was studied with an isolated, perfused hindquarters preparation. Four separate studies were conducted, and the findings were as follows. 1) Compared with New Zealand normotensive rats (NZNR), NZGH rats by 3 weeks of age clearly showed hyperresponsiveness, although the BP difference had not yet fully developed. 2) Bilateral renal denervation of NZGH at 3 weeks of age delayed the development of hypertension for 4 weeks, but did not lessen the vascular hyperresponsiveness. 3) In one-kidney, one clip renal hypertensive NZNR, vascular responsiveness was increased but remained less than that of age-matched NZGH. 4) In an F2 generation of NZGH-NZNR cross-bred rats, the average adult systolic BP was 163 mm Hg, similar to that of the NZGH parent strain; however, vascular responsiveness was reduced to an intermediate level, lower than that of NZGH but higher than that of NZNR. It is concluded that the vascular hyperresponsiveness of NZGH rats to NE is a primary characteristic that can be largely dissociated from elevated BP. (Hypertension 6: 861-867, 1984)

KEY WORDS • genetic hypertension • vascular responsiveness • hypertension • pressor responsiveness • perfused hindquarters

It has been known for many years that vascular hyperresponsiveness to vasoconstrictor agents is a characteristic of human essential hypertension and of genetic and other forms of experimental hypertension in rats. Despite extensive investigation, however, the relationship between the elevated blood pressure (BP) and the vascular hyperresponsiveness has not been fully defined. One of the fundamental questions that remains incompletely answered is whether the hyperresponsiveness is a primary difference that could be causal in the development of hypertension, or a secondary effect of the high BP.

Evidence supporting the latter interpretation is derived from studies showing that increases and decreases in BP result, respectively, in increases and decreases in the blood vessel wall-to-lumen ratio, and that such structural changes can contribute to observed differences in resistance to flow and responsiveness to vasoconstrictor agents. Evidence supporting the alternative interpretation, that vascular hyperresponsiveness may be a primary characteristic in hypertension, is found in an early study of the Dahl salt-sensitive rat and in more recent studies of the Okamoto spontaneously hypertensive rat (SHR). In these strains of genetically hypertensive rats, vascular hyperresponsiveness can be clearly demonstrated even in the absence of elevated BP.

We and others have reported that the New Zealand genetically hypertensive (NZGH) rat is also hyperresponsive to vasoconstrictor agents, but the phenomenon has not previously been extensively investigated in this strain. We undertook the present study to examine the association between BP and vascular hyperresponsiveness in the NZGH rats, utilizing pressor responses of the isolated, Krebs-perfused hindquarter preparation to norepinephrine (NE) as an index of responsiveness. We used four experimental approaches, to either minimize the BP difference between NZGH rats and their normotensive control strain (NZNR), or to generate differences in BP between rats of the same genetic background.

Methods and Materials

We studied male New Zealand normotensive (NZNR) and genetically hypertensive (NZGH) rats from the colony maintained at this institution. Rats were weaned at 21 days of age and received tap water and standard rat chow (Purina 5001; sodium, 174 mEq/kg; potassium, 282 mEq/kg) ad libitum until the day of study. They were housed in group cages, four to six rats in a cage, under conditions of controlled tempera-
tured (24°C), humidity (50%), and light (illuminated from 6:00 a.m. until 6:00 p.m.). Systolic BP (SBP) was measured by tail sphygmomanometry at weekly intervals and on the day before the acute study after the rats had been warmed at 37°C for 10 minutes.

When SBP was measured on 6 consecutive days in a randomly selected group of rats, the coefficient of variation for this method was 7.6%. In a previous study, the BP was measured in adult NZGH and NZNR rats indirectly by tail sphygmomanometry and directly by chronic femoral artery cannulation. The SBP measured by the indirect method was 193 ± 2 mm Hg in the NZGH and 131 ± 5 mm Hg in the NZNR; directly measured mean arterial blood pressure (MAP) revealed comparable differences between the two groups: 173 ± 6 mm Hg in the NZGH and 121 ± 2 in the NZHR.

Isolated, Perfused Hindquarter Preparation

Rats were weighed and anesthetized with sodium pentobarbital, 60 mg/kg, i.p. The abdomen was opened with a midline incision, and the abdominal aorta below the renal arteries was exposed. A polyethylene cannula was inserted into the aorta at the level of the lumbar arteries and advanced toward the tail until the cannula tip lay just proximal to the iliac arteries. Perfusion of the hindquarters was begun immediately with a peristaltic pump (Harvard Apparatus, Model 1203, Millis, Massachusetts). The perfusion fluid consisted of Krebs-Ringer-bicarbonate solution containing (mM): NaCl, 120; KCl, 4.8; Mg SCy7 H-O, 1.3; CaCl2, 1.2; NaHCO3, 25.2; dextrose, 6; and an artificial colloid (Ficoll, 8 g/liter, MW = 400,000, Sigma Chemical Company, St. Louis, Missouri). The solution was warmed to 38°C and bubbled with 95% O2-5% CO2. The vena cava was widely incised to facilitate perfusate outflow, and the torso was severed completely at a level just proximal to the site of cannula insertion.

The severed forequarter portion was weighed, and the initial weight of the perfused hindquarter portion was estimated from the difference between total body and forequarter weights. Perfusion pressure was monitored with an extracorporeal flow transducer (Gould-Statham, P 23, Hato Rey, Puerto Rico) connected through a side-arm cannula, and the perfusate flow rate was continuously measured using a Grass polygraph. Perfusion rates for each group were constant, selected to produce an initial perfusion pressure of 20 to 30 mm Hg, and ranged from 2 ml/min in weanling rats to 5 ml/min in adults. The perfusion rate per gram of perfused tissue was equal in groups whose responses to norepinephrine (NE) were compared.

In pilot studies, it was observed that small variations in perfusion rate and pressure did not alter the magnitude of the pressor responses to NE. After a 15-minute stabilization period, bolus injections (0.1 ml) of NE (Levophed bitartrate, Breon Laboratories Inc., New York, New York) were administered at doses ranging from 0.1 to 50 fsg at 10-minute intervals in a random sequence that was repeated, so that each duplicate dose was administered after a 1-hour interval. The average peak increase in perfusion pressure was calculated for each dose. Accumulation of edema fluid in the perfused hindquarters was evident, but it did not differ among the various comparison groups and did not affect the pressor response to NE. In pilot studies, pressor responses to repeated 10 pg doses did not vary significantly over a period of 120 minutes. In addition, responses to the randomly sequenced duplicate NE doses did not differ between the first and second administrations.

Initial hindquarter vascular resistance was calculated as the ratio of perfusion pressure to perfusion rate per 100 g hindquarter tissue, and is expressed as mm Hg/mL/min/100 g tissue. The perfusion pressure recorded just prior to administration of the first NE dose was used for calculations. Baseline perfusion pressure increased by 10 to 20 mm Hg over the 2-hour period of study. Hindquarter temperature was monitored from a thermistor inserted into the rectum; it remained constant at 37° to 38°C.

Experiment 1

The objective of this protocol was to compare the pressor responsiveness of NZGH and NZNR prior to, during, and after the development of adult level hypertension in the NZGH. Rats were studied at 3 to 4 weeks of age (eight NZGH, eight NZNR), 7 to 8 weeks of age (four NZGH, four NZNR), and 15 to 18 weeks of age (eight NZGH, eight NZNR).

Experiment 2

The objective of this protocol was to determine the effect of a sustained reduction in BP on the pressor responsiveness of NZGH rats. As we have previously reported, bilateral renal denervation of weanling NZGH retards the development of hypertension for a period of several weeks, and this procedure was used to produce a sustained BP reduction in the present study. NZGH rats were subjected to bilateral renal denervation (n = 5) or sham operation (n = 4) at three weeks of age. The rats were anesthetized with ether, and incisions were made in both flanks. The renal arteries were first stripped of all visible nerve fibers and adventitial tissue from the aorta to the bifurcation of the renal artery, and then wrapped for 10 minutes with a thread soaked in 10% phenol solution. Incisions were closed, the rats were allowed to recover, and the pressor responsiveness was determined 4 weeks after surgery.

Experiment 3

The objective of this protocol was to determine the effect of a sustained elevation of BP on the pressor response of NZNR. Following the method of Brooks et al., we removed the right kidney and placed a silver clip (0.2 mm i.d.) on the left renal artery of 7-week old
NZNR \((n = 7)\). At 8-to-10 weeks after surgery, we determined the pressor responses of the perfused hindquarters and compared them with those of age-matched NZNR and NZGH.

Experiment 4
The objective of this protocol was to determine whether the hypertension and vascular hyperresponsiveness of the NZGH rats could be dissociated by cross-breeding with the NZNR. The initial cross between a male NZGH and a female NZNR produced two litters of eight to 10 pups. From these, three sibling pairs were randomly selected as breeders; from three resultant litters, 12 male F\(_2\) pups were randomly selected, and BP was measured weekly. Pressor responses were determined at 16 weeks of age and compared with those of age-matched NZGH and NZNR.

Data Analysis
Two-group comparisons were made with Student’s \(t\) test for unpaired values. Multiple group comparisons were made by analysis of variance, followed by the Neuman-Keuls test for significance. A \(p\) value of less than 0.05 was taken to indicate statistical significance. Values throughout the text, figures, and tables are expressed as the group mean value ± the standard error of the mean (SEM).

Results
Comparisons between Weanling, Adolescent, and Adult NZGH and NZNR
The SBP of NZGH progressively increased over the 15-week period of observation. In contrast, the SBP in the NZNR reached adult levels by 7 to 8 weeks of age and did not increase thereafter. As a result, the SBP difference was less than 20 mm Hg at 3 weeks of age and progressively increased to almost 40 mm Hg in the adult rats. Despite the continuing increase in both absolute BP of the NZGH and in the BP difference between NZGH and NZNR, differences in the pressor responses of the perfused hindquarters were qualitatively similar for the three age groups (Figure 1). The NZGH exhibited approximately a twofold greater pressor response than the NZNR. Over the range of doses of NE from 1 to 50 \(\mu g\), the slopes of the dose-response relationships for the NZGH rats exceeded those of the corresponding NZNR in all age groups (15-to 18-week-old rats: NZGH = 4.3 ± 0.3 mmHg/\(\mu g\) NE, NZNR = 2.0 ± 0.2; 7- to 8-week-old rats: NZGH = 3.5 ± 0.2; NZNR = 1.4 ± 0.2; 3- to 4-week-old rats: NZGH = 2.5 ± 0.2, NZNR = 1.4 ± 0.2; \(p < 0.01\) in all cases).

Initial hindquarters vascular resistance of the 15- to 18-week-old NZGH (4.5 ± 0.3 mm Hg/ml/min/100 g tissue) was slightly, but significantly, higher than that...
of the NZNR (3.4 ± 0.3 mm Hg/ml/min/100 g tissue; \( p < 0.01 \)), but did not differ significantly between either the 7- to 8-week-old NZGH (12 ± 2) and NZHR (8 ± 2 mm Hg/ml/min/100 g tissue) or the 3- to 4-week-old NZGH (153 ± 14) and NZHR (139 ± 21 mm Hg/ml/min/100 g tissue). In preliminary studies in adult rats with progressive increases in perfusion rate, perfusion pressure was observed to increase to a greater extent in the hindquarters of NZGH than in those of NZNR (data not shown).

**Effect of Sustained BP Reduction in NZGH**

Bilateral renal denervation of NZGH at 3 weeks of age retarded the progressive rise in BP and delayed the development of hypertension for a period of 4 weeks. As a result, the SBP of the denervated rats was lower than that of the sham-operated NZGH for the entire 4-week period. Just prior to study, the SBP of the denervated NZGH was 25 mm Hg lower than that of the sham-operated NZGH. However, as shown in Figure 2, despite this prolonged BP reduction to approximately normotensive levels, the pressor response of the perfused hindquarters of the renal denervated NZGH was not altered, being indistinguishable from that of the sham-operated NZGH.

**Effect of Sustained BP Elevation in NZNR**

Following renal artery clipping, the BP of the NZNR rose progressively for 3 to 5 weeks and reached a maximum level that was sustained until the acute study. On the day prior to the acute study, the SBP of the clipped-artery NZNR averaged 222 ± 10 mm Hg, which was greater than the SBP of either the NZNR control rats (123 ± 6 mm Hg) or the NZGH (166 ± 2 mm Hg). Thus, the clipped NZNR rats exhibited hypertension of greater severity than that of the NZGH, but of a shorter duration.

As shown in Figure 3, the perfused hindquarter response to NE was increased for the hypertensive NZNR, in comparison with the normotensive NZNR. However, the response of the hypertensive NZNR remained substantially less than that of the NZGH. The initial hindquarter resistance of the renal-clipped NZNR (11.6 ± 1.2 mm Hg/ml/min/100 g tissue) was

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**FIGURE 2.** Systolic blood pressure (SBP) prior to acute study and perfused hindquarter perfusion pressure responses (hPP) to bolus injections of norepinephrine (NE) of 7-week-old NZGH rats that were bilaterally renal denervated (X) or sham-operated (solid circles) at 3 weeks of age. Vertical bars indicate SEM.

**FIGURE 3.** Systolic blood pressure (SBP) prior to acute study and perfused hindquarter perfusion pressure responses (APP) to bolus injections of norepinephrine (NE) of 16- to 18-week-old NZGH (closed circles), normotensive NZNR (open circles), and hypertensive (one-kidney, one clip) NZNR (open triangles). * indicates \( p < 0.05 \).
more than twice that of the age-matched NZGH (4.5 ± 0.3 mm Hg/ml/min/100 g tissue, \( p < 0.05 \)). The slope of the dose-pressor response relationship of the clipped NZNR (2.6 ± 0.4 mm Hg/\( \mu \)g NE) was less than that of the NZGH (4.3 ± 0.5 mm Hg/\( \mu \)g NE, \( p < 0.05 \)) but not different from that of the normal NZNR (2.0 ± 0.2 mm Hg/\( \mu \)g NE).

### Blood Pressure and Pressor Response of Crossbred Rats

The SBP at 4, 8, and 17 weeks of age are shown in Figure 4 for NZGH, NZNR, and the \( F_2 \) GH-NR crossbred rats. Separation of the pressures of NZGH and NZNR was significant by 4 weeks of age (NZGH = 114 ± 7; NZNR = 96 ± 5 mm Hg; \( p < 0.05 \)) and was clearly evident at 8 and 17 weeks of age. Pressures in the \( F_2 \) GH-NR rats overlapped those of both the NZGH and NZNR, ranging from normotensive to quite hypertensive at all ages. For the \( F_2 \) GH-NR rats, the average SBP at 4, 8, and 17 weeks of age was 136 ± 9, 146 ± 7, and 163 ± 5 mm Hg, respectively. Thus, the average pressure of adult \( F_2 \) GH-NR rats was almost identical to that of the adult NZGH (162 ± 4 mm Hg).

However, as shown in Figure 5, pressor responses of the perfused hindquarters of the \( F_2 \) GH-NR rats were intermediate to those of the NZGH and NZNR parent strains, being significantly greater than those of the NZNR but significantly less than those of the NZGH. The initial hindquarter resistance of the \( F_2 \) GH-NR rats (6.6 ± 1.0 mm Hg/ml/min/100 g tissue) was greater than that of the age-matched NZNR (3.4 ± 0.3 mm Hg/ml/min/100 g tissue, \( p < 0.01 \)), but was not significantly different from that of the corresponding NZGH (4.5 ± 0.3 mm Hg/ml/min/100 g tissue). The slope of the dose-pressor response relationship of the \( F_2 \) GH-NR rats (2.5 ± 0.4 mm Hg/\( \mu \)g NE) was intermediate to that of the NZNR (2.0 ± 0.2 mm Hg/\( \mu \)g NE) and the NZGH (4.3 ± 0.5 mm Hg/\( \mu \)g NE).

Responses of the \( F_2 \) GH-NR were further evaluated to determine the degree of association between the BP measured the day before the acute study and the hindquarter responses to intermediate doses of NE. As shown in Figure 6, significant correlations between BP and pressor response to NE were demonstrated for 10 and 20 \( \mu \)g doses, and the relationship approached significance for the 5 \( \mu \)g dose. However, \( r^2 \) values ranged from 0.22 to 0.32, which indicated that not more than 30% of the variance of either variable could be accounted for by the variance of the other.

### Discussion

This study demonstrates that vascular hyperresponsiveness of the New Zealand genetically hypertensive rat is a primary characteristic that can be largely dissociated from the high BP also characteristic of this strain of rats. Findings supporting this conclusion are the following. First, hyperresponsiveness was already clearly evident by 3 weeks of age, at a time when the BP of the NZGH only slightly exceeded that of the NZNR. Second, interrupting the development of hypertension in weanling NZGH by bilateral renal denervation caused the BP to remain at near-normotensive levels.
levels for 4 weeks. This extended BP reduction did not reduce pressor responsiveness, however. Third, while pressor responses of NZNR made hypertensive by renal artery clipping exceeded those of normotensive NZNR, they remained substantially less than those of NZGH. Fourth, although the average BP of F$_2$ GH-NR crossbred rats was not different from that of NZGH, the pressor responsiveness was clearly reduced. Regression analysis indicated that the interdependence of F$_2$ GH-NR BP and pressor response was not more than 30%.

Studies in other strains of genetically hypertensive rats also support the conclusion that pressor hyperresponsiveness is a primary characteristic, rather than a secondary effect of high BP. In Dahl sodium-sensitive (S) rats, which develop hypertension only on a high-salt diet, hyperresponsiveness was found to precede the development of hypertension. These S rats were hyperresponsive to various pressor agents regardless of diet or BP.

Okamoto SHR treated with timolol in utero and from birth did not develop hypertension, but did exhibit characteristic aortic ring hyperresponsiveness. The SHR that spontaneously failed to develop hypertension were found to have hindquarter and mesenteric artery constrictor responses that were greater than those of the normotensive control-strain rats and were not less than those of SHR that developed hypertension as expected. Chronically shielding SHR femoral arteries from exposure to high BP did not alter the characteristic contractile responses of the vascular smooth muscle. Like the 3-week-old NZGH in the present study, 3-week-old SHR are clearly hyperresponsive to vasoconstrictor agents when compared to age-matched normotensive control-strain rats. At 3 weeks of age, the SHR BP is not different from that of rats of the normotensive control strain, in contrast to the NZGH BP, which is already slightly but significantly elevated by the first few days after birth. Recently, from an extensive study of backcross rat populations derived from an original cross between SHR and Dahl salt-resistant (R) rats, Rapp has provided evidence that a distinct autosomal locus controls the vascular smooth muscle responsiveness to constrictor stimuli.

While the principal aim of the present study was to assess in NZGH rats the degree to which hindquarter hyperresponsiveness to NE is related to BP, an equally important question needs to be answered. If, as proposed on the basis of our findings and those described above, vascular hyperresponsiveness is a primary characteristic of rats of various genetically hypertensive strains, what is the contribution of this characteristic to the development of hypertension? It is clear that it is possible for a rat of a genetically hypertensive strain to be hyperresponsive without developing hypertension, since renal-denervated NZGH exhibited characteristic hyperresponsiveness but were not hypertensive. Similarly, in Dahl S rats and SHR, vascular hyperresponsiveness does not guarantee that rats will develop high BP. However, although it may be concluded that vascular hyperresponsiveness by itself is not sufficient to cause hypertension, it would not be correct to conclude from these data that vascular hyperresponsiveness does not contribute to the development of hypertension.

Studies in both the SHR and the NZGH rat have shown that BP is determined by the combined influences of multiple loci; while the development of hypertension may be predominately attributed to a single, specific locus, other loci may contribute through modulatory systems. Definitive quantitative data are not yet available regarding the possible contribution of vascular hyperresponsiveness to genetic hypertension. Based on distribution patterns for hyperresponsiveness and BP, Rapp has suggested that there exists a discrete locus that regulates vascular responsiveness, and he has estimated that the BP of a rat is elevated by 15 mm Hg by homozygosity for the characteristic. Although the design of our study does not permit anything more than speculation on this point, our findings are least consistent with those of Rapp in the R-SH rats.

We found that the range of BP in the F$_2$ GH-NR rats was 45 mm Hg, and regression analysis of the BP and hindquarter responsiveness data indicated that, at

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**FIGURE 6.** Individual values for systolic blood pressure (SBP) just prior to the acute study, and perfused hindquarter perfusion pressure responses (&PP), to 5 y. g (Panel A), 10 y.g (Panel B), and 20 yg (Panel C) bolus injections of norepinephrine, of 16-to 18-week-old F$_2$ generation GH-NR crossbred rats.
most, 30% of the variance in BP, or 15 mm Hg, might be determined by the variance in vascular responsiveness. The mechanism underlying the increased responsiveness to vasoconstrictor agents of vascular tissue of genetically hypertensive rats remains incompletely defined. The hyperresponsiveness appears to be nonspecific with respect to pressor agents. The SHR hindquarters exhibit increased pressor responses to diverse agents, including NE, 5-hydroxytryptamine, and potassium chloride, and we have reported that intact, chronically cannulated NZGH are hyperresponsive to the pressor actions of both angiotensin II and vasopressin. Folkow and colleagues studied perfused SHR hindquarters responses to NE; they found that the slope of the dose response curve was increased by about 50% hindquarter vasculature could be accounted for by an increase in wall mass of the contractile vessels, with encroachment of the wall on the vessel lumen even at rest or during vasodilation. Based on analysis of these findings, they concluded that the hyperresponsiveness of the SHR hindquarters vasculature could be accounted for by an increase in wall mass of the contractile vessels, with encroachment of the wall on the vessel lumen even at rest or during vasodilation.

Findings in our present study of NZGH hindquarters are in part similar to the findings described above in that, in adult rats, the NZGH exhibited approximately a twofold increase in the slope of the NE-pressor response relationship, and basal resistance of the hindquarters to perfusate flow was increased by about 30% and that hindquarter resistance to perfusate flow was increased under resting conditions or during maximal vasodilation. Based on analysis of these findings, they concluded that the hyperresponsiveness of the SHR hindquarters vasculature could be accounted for by an increase in wall mass of the contractile vessels, with a factor in vascular hyperresponsiveness of the NZGH, as proposed for the SHR. It should be noted, however, that the slope of the NE-pressor response relationship was also increased in young NZGH rats exhibiting little difference in BP, and was not decreased in renal-denervated NZGH in which BP was reduced for a period of several weeks. From these findings, it appears that if increased contractile vessel wall mass plays a role in vascular hyperresponsiveness of the NZGH rat, the alteration in vascular anatomy may not be dependent on absolute BP levels. Rather, the vascular alteration may be a genetically determined characteristic of rats with this form of genetic hypertension.

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