Sympathetic Nerve Activity and Blood Pressure in Normotensive Backcross Rats Genetically Related to the Spontaneously Hypertensive Rat

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SUMMARY The genetic basis of hyperactivity of the sympathetic nervous system (SNA) in spontaneously hypertensive rats (SHR) was assessed by measuring SNA in animals derived from a backcross (BC) breeding program designed to isolate single gene differences causing changes in blood pressure. Selective breeding of the male hypertensive rats with inbred normotensive female Wistar/Lewis rats yielded progeny with a range of blood pressures, but whose group mean pressures were lower than the group mean pressures of the original SHR. Progressive generations had progressively lower group mean pressures. There was a positive correlation between SNA and mean arterial pressure in BC rats. These results indicate that the genetic defect in SHR may be an abnormality in SNA, and the hypertension in these animals is a secondary result of this primary defect. Baroreceptor function was also assessed in SHR and in BC rats. In young (8 to 24 weeks old) SHR, baroreceptor function was similar to that in BC rats, whereas SNA was markedly increased. Only in older (24 to 40 weeks old) SHR was there an abnormality in the gain of baroreceptors. The development of hypertension in SHR therefore appears to be due to increased SNA resulting from a defect in the central nervous system. Changes in baroreceptor function are secondary to the hypertension and occur after the hypertension is established. (Hypertension 1: 598-604, 1979)

KEY WORDS • spontaneous hypertension • sympathetic nervous system • backcross rat • genetics • blood pressure

EXCESSIVE activity of the sympathetic nervous system has been shown to be a significant causative factor in the hypertension of the Okamoto strain of the spontaneously hypertensive rat (SHR). Directly measured sympathetic nerve activity (SNA) is markedly increased in the SHR compared to normotensive Wistar rats, and surgical or pharmacological abolition of SNA leads to greater reductions in blood pressure in the SHR. The mechanisms responsible for the increased SNA, which results in hypertension in the SHR, are unknown. Two proposed mechanisms are adaptive changes in arterial baroreceptors or neurochemical changes in the central nervous system's (CNS) sympathetic centers, both of which have been postulated to result in increased SNA and the consequent hypertension.

Although the SHR has been widely used as a model of essential hypertension, the results and conclusions derived from studies using this animal have been undermined by questions of the appropriateness of the control rat used in the experiments. Most investigators agree that the Kyoto Wistar strain of normotensive rats, the parent strain of the SHR, is an adequate control animal. However, their usefulness has been modest because of their limited availability. To provide another control strain genetically related to the SHR, one of the authors (P.L.Y) initiated a backcross breeding program in 1972 to develop hypertensive and normotensive lines of rats from a cross between SHR and a normotensive, inbred strain (Wistar/Lewis). In addition to providing another appropriate control for the SHR, these backcross (BC) normotensive rats also allowed us to further explore the role of excessive SNA in the hypertensive animal and the mechanisms of the elevated SNA.

The purpose of the present study was to determine by direct measurements whether the blood pressure reduction in the BC rat was accompanied by reductions in SNA, and to analyze the role of the arterial baroreceptors and the CNS in the generation of elevated SNA. Our findings indicate that the reduction of blood pressure in the BC rats was associated with a diminution of CNS sympathetic outflow. Arterial baroreceptor control of SNA in young SHR and BC rats was similar. However, in older SHR a
significant loss of baroreceptor control of SNA occurred. These results support our previous conclusions that the hyperactive sympathetic nervous system in the young SHR is central in origin. Adaptive changes in baroreceptor inhibitory control of SNA occur after the initial rapid increase in blood pressure and SNA. Therefore, the CNS appears to be the primary origin of the hyperactive sympathetic nervous system in young SHR.

Methods

Experiments were conducted on groups of male SHR (Okamoto strain, Cox Laboratory Animal Supply) and on normotensive and hypertensive male BC rats raised in our own facility. The BC rats were developed as follows: hypertensive male SHR were mated to normotensive female Wistar/Lewis rats producing the F₁ generation (fig. 1). The male F₁ rats were then mated to female Wistar/Lewis rats producing the first backcross generation (BC₁). In each successive backcross generation the males selected for breeding had the highest average blood pressures. The male rats of the fifth and sixth BC generation were used in this study. The systolic blood pressure in conscious rats was measured by the tail-cuff plethysmographic method.

All rats were anesthetized with sodium pentobarbital (20 mg/kg, i.p.), and a light level of anesthesia was maintained with supplemental doses of 2.5 mg/kg given intravenously as needed. Arterial pressure was measured at the level of the terminal aorta using a pressure transducer (Model P23-Dd, Statham Instruments, Oxnard, CA) connected to a heparinized saline-filled polyethylene tube (PE-60, thin walled). Central arterial pressure was measured from the left brachial artery using the same type transducer and catheter system. Mean arterial pressure (MAP) was derived using an electronic averaging circuit. The right femoral vein was cannulated (PE-60 tubing) for administration of supplemental doses of anesthetic. Heart rate (HR) was determined by counting the number of arterial pressure pulsations per minute. Sympathetic nerve activity was measured from the renal nerves. A left lateral incision was made to expose the kidney, its blood supply and sympathetic nerves. A small bundle of postganglionic nerves was separated from the nerve plexus adjacent to the renal artery using microscopic assistance (Model 644051, Carl Zeiss). The connective tissue coverings were removed and the nerve was placed across a pair of fine stainless steel electrodes. The nerve bundle and electrodes were bathed in a pool of 37°C mineral oil to prevent tissue drying. The efferent electroneurograms were recorded and processed as previously described. Briefly, the efferent signals were recorded by means of an AC differential preamplifier (Model P-15, Grass Instrument Co., Quincy, MA) with a time constant of 3 msec. The amplified nerve spikes were displayed on an oscilloscope (Model R103N, Tektronix, Inc., Beaverton, OR) for visualization and photographing (Tektronix Camera, Model C-12). These signals were further amplified, then rectified with a full-wave rectifier circuit and integrated continuously with an RC integrator (time constant 20 msec). The integrated signals were averaged using an RC network with a time constant of 1 second. The nerve data presented in this paper are expressed as mean SNA and are calibrated in microvolts (µV) above noise level. The noise level was determined by shorting the input electrodes, and this value, for each experiment, was electronically subtracted from the electroneurogram by adjusting the zero bias level on the recorder. All analog and electronically averaged signals were recorded on a Beckman Type R Dynograph (Beckman Instruments, Inc., Fullerton, CA). To summarize for graphical presentation, mean SNA was determined by measuring the area under the electronically derived curve over a period of 10 to 30 seconds. Mean arterial pressure and HR were also measured during this interval. Data collection began 25 to 30 minutes after the experimental setup was complete.

To compare the levels of MAP, SNA and HR of the normotensive BC rat with those of the SHR during the development and maintenance phases of hypertension, groups of six rats at 8, 16, 24 and 40 weeks of age were tested in the fifth backcross (BC₅) generation. To determine the SNA-MAP relationship in the BC rats, 10 16-week-old rats were randomly selected (no selection based on MAP) from the BC sixth generation (BC₆) and tested.

To determine if the blood pressure reduction in the BC rats was due to an increased sensitivity of the arterial baroreceptor or to a central reduction of SNA, the gain of the baroreceptor-SNA system in the different age groups was measured. In these experiments MAP was abruptly increased 40–50 mm Hg and held at this level for 15–20 seconds. This procedure was done three times on each rat over a period of 20 minutes. The rise in MAP was induced by occluding the abdominal aorta with a snare occluder. The reflex inhibition of SNA was measured during the interval between 5 and 15 seconds of occlusion. The gain of the baroreceptor-SNA system was calculated from the ratio of the percent SNA decrease to that of the MAP rise. Six animals in each age group for both SHR and BC₅ rats were tested, and the mean of three occlusion procedures was calculated as the baroreceptor-SNA gain for each animal. Mean gain for each age group was calculated from the individual rat mean values.

Statistical differences between the mean values of the SHR and BC groups were evaluated with a two-sample t-test. Linear regression analysis was used to test the SNA-MAP relationship in the BC₆ group. All data presented are expressed as means ± SEM unless otherwise stated.

Results

Effect of Selective Backcrossing on Blood Pressure

Mating the most hypertensive males of the F₁ generation, and those of each successive BC genera-
tion, with normotensive female Wistar/Lewis rats yielded progeny with reduced blood pressures. Each generation of BC rats generally had progressively lower blood pressures (fig. 1). Group mean systolic blood pressure was lowered from 170 mm Hg for the F, generation to 128 mm Hg for the BC, generation. There was a considerable range of pressures, which shows segregation of the genes affecting blood pressures among these rats. From the normotensive (systolic pressure below 130 mm Hg) BC, rats, control animals for the SHR were obtained.

**Changes in Mean Arterial Pressure, Sympathetic Nerve Activity and Heart Rate in SHR and Backcross Rats with Age**

With age, MAP and SNA increased in the SHR as previously reported. The group mean MAP value was 116 ± 3.4 mm Hg at 8 weeks of age and increased to 170 ± 7.6 mm Hg at 40 weeks (fig. 2). The greatest MAP change occurred between the 8th and 24th weeks and pressure changed only slightly in the subsequent weeks. Group mean SNA increased with age in the SHR paralleling the pressure increase (fig. 3). Mean SNA increased from 24 ± 3.3 μV at 8 weeks of age to 68 ± 7.9 μV at 40 weeks. The major SNA change occurred during the rapid pressure developing phase; thereafter SNA increased less rapidly.

Mean MAP in the BC, rats was significantly less (p < 0.01) than that of each SHR age group tested (fig. 2). At 8 weeks of age, MAP was 94 ± 2.7 mm Hg, at 16 weeks 105 ± 4.6 mm Hg and, subsequently, pressure increased only slightly. Mean SNA in these animals was also significantly less (p ≤ 0.01) than that of the SHR at the ages tested (fig. 3). At 8 weeks of age, mean SNA was 14 ± 3.4 μV, at 16 weeks 18.6 ± 4.6 μV, and this value remained constant in older age groups.

Heart rate decreased slightly as a function of age in both the SHR and BC rats; there was no significant difference in HR between strains at any age tested.
**Figure 2.** Mean arterial pressure levels of groups of six spontaneously hypertensive (SHR) and normotensive backcross (BC₅) rats of different ages.

**Figure 3.** Mean sympathetic nerve activity levels of groups of six spontaneously hypertensive (SHR) and normotensive backcross (BC₅) rats of different ages.

### Relationship Between Sympathetic Nerve Activity and Mean Arterial Pressure in Backcross Rats and SHR

From figures 2 and 3 it is apparent that a positive relationship between SNA and MAP exists for the SHR, as we have previously reported.¹,² No such relationship was apparent for the control rats which were selected because of their normal blood pressures. However, if BC₅ rats were randomly selected and studied (there was a range of blood pressures for these rats; fig. 1), a positive correlation between SNA and MAP became apparent in these rats as well (fig. 4). In these rats, MAP ranged from 98 to 163 mm Hg and mean SNA from 17 to 66 μV.

### Influence of Arterial Baroreceptor Stimulation on SNA in SHR and Backcross Rats of Different Ages

With an abrupt increase in MAP (MAP change = 46.3 ± 3.7 mm Hg in both SHR and BC₅ rats), mean SNA was reflexly inhibited. However, the magnitude and duration of the inhibitory response was different between the SHR and BC rats. In the BC₅ rats, the magnitude and the duration of the reflex inhibition of SNA remained fairly constant in the different age groups (fig. 5). By contrast, the efficacy of the reflex inhibition of nerve activity decreased with age in the SHR (fig. 6). In the 24- and 40-week-old rats, the magnitude of reflex inhibition of SNA in response to the same amount of induced hypertension was less, and the duration of inhibition was shortened (fig. 6). The average mean SNA reduction in the 8-, 16-, 24- and 40-week-old SHR groups were 22.0 ± 0.6, 43.1 ± 3.7, 32.0 ± 3.6 and 30.2 ± 2.6 μV respectively; whereas those of the same age BC₅ groups were 14.0 ± 0.4, 18.0 ± 0.7, 16.6 ± 1.4 and 17.3 ± 1.8 μV for the respective groups. Obviously, both the basal levels and reflex reductions in mean SNA were greater in the SHR groups. To further analyze the baroreceptor inhibitory effect on mean SNA, the gain of the baroreceptor-SNA system was determined. The gain was calculated from the ratio of the percent decrease in SNA to that of MAP rise. Such an analysis showed that the gain of this system was almost identical for the 8- and 16-week-old SHR and BC rats (fig. 7). The gain was 2.68 ± 0.28 in the 8-week-old SHR and BC rats, and 2.46 ± 0.30 in the 16-week-old SHR and BC rats. The difference in gain between the 24- and 40-week-old SHR and BC rats was also significant (p < 0.01). A slight but insignificant gain reduction was observed in the older BC rats as a function of age (2.56 ± 0.32 at 8 weeks vs 2.15 ± 0.20 at 40 weeks).
Discussion

The results of the present study indicate that the SHR is a genetic model of hypertension in which the sympathetic nervous system is hyperactive compared to that of genetically related normotensive rats (fig. 3). The fact that SNA and MAP were highly correlated in a population of rats whose genes are segregating strongly suggests a cause and effect relationship between these two physiological parameters (fig. 4). Alternative explanation, other than a cause and effect relationship between SNA and MAP, is that increased SNA was secondary to the hypertension. The results of the baroreceptor experiments, however, suggest that this explanation is not correct because in young SHR, baroreceptor function was normal at a time when SNA was markedly increased (fig. 7). In other words, baroreceptor "resetting," which might secondarily lead to increased SNA, did not occur early in the development of hypertension. Thus, the SHR may in fact represent an animal model of hyperactive sympathetic nervous system, and the hypertension may be a secondary result of this primary genetic defect.

Further evidence for a cause and effect relationship between SNA and MAP is presented by the finding that backcrossing reduced group mean systolic pressures in all BC generations (fig. 1) and SNA currently in the BC$_6$ group (fig. 3). Additionally, SNA and MAP were found to increase simultaneously as a function of age in the SHR (figs. 2 and 3), and neither of these parameters increased in BC$_6$ rats selected for normal blood pressure. It thus appears that excessive SNA is a causative factor for the development and maintenance of hypertension as previously reported.

The pressures of the rats in each successive BC generation ranged from normotensive to hypertensive levels, indicating that the genes affecting blood pressure were segregating among the BC rats (fig. 1). If the BC$_6$ rats had been selected randomly instead of screened by the tail-pressure method they would not have served as good controls for the SHR. Since we knew the systolic pressures of these BC$_6$ rats, these experiments cannot be designated as a blind study; however, testing of the randomly selected BC$_6$ generation (fig. 4) was done in a blind fashion, since we did not know the systolic pressures in this group.

In the interpretation of the voltage level from the multifiber preparation it should be stressed that the simple integration method used in this study is not a measure of nerve frequency. This method transforms the recorded nerve spikes into voltage. The major limitation of the method is that it does not accurately reflect the number of active fibers in the nerve bundle from which the recording is made. For example, larger nerve spikes will contribute more than smaller ones to the total measured voltage. This method provides no quantitation of number of nerve spikes, except for the original electoneurogram displayed on an oscilloscope and photographed at a high sweep speed. Another way to measure the level of nerve spike frequency is to transform each spike into standard pulses and integrate these. The resulting output voltage will reflect the frequency of the nerve bundle. We have used both methods to quantitate the basal frequency level in a small group of 12-week-old SHR and WKY rats ($n = 12$). By both methods we find that the mean frequency level in the SHR is significantly greater than those of normotensive rats. However, we do not know the actual number of active fibers, and without expensive computer systems with pulse discriminators we cannot obtain such information.

Additional insight into the mechanisms responsible for the excessive SNA and resultant hypertension in the SHR is also provided by the results of this study. Excessive SNA appears in young SHR (8 and 16 weeks old) during the development of hypertension (figs. 2 and 3). By contrast, in the young SHR, the gain of the baroreceptor-sympathetic system was similar to that of the BC$_6$ normotensive rats, indicating that resetting of the baroreceptors was not responsible for the elevated SNA (fig. 7). This suggests that central mechanisms are responsible for the increased SNA in the young SHR during the development of hypertension. The precise location(s) within the CNS of the abnormalities leading to excessive SNA remains unknown, although several recent studies suggest involvement of the hypothalamus and/or the brain stem. Similarly, the neurochemical basis of the CNS abnormality is unknown. Several studies...
suggest, however, an abnormality in central adrenergic pathways, which results in either excessive activity of excitatory centers or inadequate activity of inhibitory centers. Saavedra et al. have recently shown a reduction in dopamine-beta-hydroxylase activity and norepinephrine levels in specific nuclei of the hypothalamus, as well as abnormally high activity of phenylethanolamine-N-transferase in the brain stem of the SHR. We have recently demonstrated that L-dopa, presumably by increasing catecholamine levels in certain areas of the brain, is a potent depressor agent in the SHR.

In the older SHR (24 to 40 weeks), baroreceptor dysfunction was present (figs. 6 and 7). In these rats, therefore, loss of baroreceptor gain may have contributed to the excessive SNA, and this factor as well as a CNS abnormality and peripheral vascular morphological changes may account for the maintenance of hypertension. The specific baroreceptor group, aortic or carotid, that was defective in the

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**Figure 5.** Influence of age on mean arterial pressure (MAP), mean sympathetic nerve activity (SNA), reflex inhibition of SNA, and the gain (G) of the baroreceptor-sympathetic system in backcross rats (BCr).

**Figure 6.** Influence of age on mean arterial pressure (MAP), mean sympathetic nerve activity (SNA), reflex inhibition (SNA), and the gain (G) of the baroreceptor-sympathetic nerve system in spontaneously hypertensive rats (SHR).
animals studied was not determined. Published studies of isolated baroreceptor systems suggest that the defective arterial baroreceptor system in the SHR is predominantly the aortic group. In 16- and 24-week-old SHR, the carotid sinus system was shown to have a normal firing frequency and sensitivity, and was reset to a new pressure threshold. In other studies, however, the firing frequency of the aortic baroreceptors was reduced in the SHR. The aortic baroreceptors were also reset. The cause of this change in firing frequency may be hypertrophy of the vasculature in the area of the aortic baroreceptors, the hypertrophy being secondary to the sustained hypertension. If this were true, the decreased gain in baroreceptor control of SNA may be essentially a secondary result of the hypertension and have no primary causal role, at least in young SHR, in the elevated SNA. In older SHR, however, this reduced sensitivity of the baroreceptors would play a permissive role in the hypertension. In any case, it appears that resetting of the baroreceptors is not a primary cause of the development of hypertension in the SHR.

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References


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