Contribution of the Sympathetic Nervous System to Vascular Resistance in Conscious Young and Adult Spontaneously Hypertensive Rats

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SUMMARY Although evidence exists for exaggerated sympathetic nervous system activity in spontaneously hypertensive rats (SHR), there are no studies in conscious animals that directly demonstrate that this increased activity is functionally involved in the elevated vascular resistance of these animals. In our present study, 8-week-old and 13-week-old SHR and Wistar Kyoto controls (WKY) were chronically instrumented with arterial and venous catheters and miniaturolized pulsed Doppler flow probes on the renal and mesenteric arteries and lower abdominal aorta. While the rats were conscious and unrestrained, hexamethonium was administered intravenously to block sympathetic nervous system transmission. Prior to hexamethonium, the mean arterial pressure of young SHR and WKY averaged 123 ± 5 and 109 ± 4 mm Hg respectively (p < 0.05), while adult SHR and WKY averaged 159 ± 7 and 128 ± 3 mm Hg respectively (p < 0.05). Hexamethonium produced an equivalent fall in arterial pressure of young SHR (−32%) and WKY (−30%) and adult SHR (−39%) and WKY (−41%). Vascular resistance was reduced by hexamethonium in the kidney, gut, and hindquarters, but the percent changes were not significant between SHR and WKY. These data suggest that, in both young and adult SHR, vascular resistance and arterial pressure are sustained at elevated levels by some other mechanism than neurally-derived vasoconstrictor tone. (Hypertension 2: 408–418, 1980)

KEY WORDS • genetic hypertension • blood pressure • sympathetic nervous system • vascular resistance • angiotensin response • norepinephrine response • conscious rat • hexamethonium

THE contribution of the sympathetic nervous system to the elevated pressure of the Okamoto-Aoki strain of spontaneously hypertensive rats (SHR) has been the subject of extensive controversy. Hypertension develops in these inbred rats without experimental intervention.1 Central neurogenic mechanisms have been implicated by studies showing hyperreactivity of blood pressure (BP) to environmental stimuli. Chronic stress,2 graded alerting stimuli,3 immobilization,4 and elevated temperature5 have been shown to provoke a greater increase in the BP of SHR than of normotensive Wistar-Kyoto rats (WKY). Conversely, deprivation of sensory stimuli retards the development of hypertension.6,7 Although no clear pattern relating to pathogenesis exists, many investigations have shown altered neurotransmitter levels and enzyme activities in the central nervous system at various stages in the development of hypertension.4,5 Additional deple-
tone, and to avoid the confounding effects of anesthesia, experiments were performed on conscious rats instrumented chronically for recording arterial BP and vascular resistance in three major vascular beds. Sympathetic transmission was blocked pharmacologically using ganglionic blockade. The results of this study do not appear to support the hypothesis that neurogenic vasoconstrictor tone is responsible for the difference in arterial BP between SHR and WKY.

Methods

Animals

Male SHR and WKY rats from inbred colonies were maintained under identical conditions in our laboratory. Both were offspring of brother-sister matings with the SHR of the 46th generation and the WKY of the 21st generation when traced back to the original pairing at NIH of rats from the Okamoto-Aoki strain. Two age groups were studied, 8-week-old rats, weighing 150-200 g, and 13-week-old rats, weighing 300-350 g.

Chronic Instrumentation

Two to 3 days before the experiments were conducted, the rats were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and were placed on a heated pad to maintain body temperature. A midline laparotomy was performed. After carefully isolating the superior mesenteric artery, the left renal artery, and the lower abdominal aorta just distal to the origin of the left renal artery, small pulsed Doppler flow probes were placed on each of the vessels. A complete description of these flow probes and their construction is found elsewhere. The wires from each probe were led subcutaneously to a small plug which was cemented to the skull of the rat. The rats were anesthetized lightly with ether. A polyethylene catheter (PE 10) filled with heparinized saline was placed into the abdominal aorta via the left femoral artery. This catheter was connected to a short section of PE 50 cannula which in turn was connected to a PE 90 cannula using heat fused joints. A PE 50 catheter was placed in the right jugular vein for intravenous injections. In the 3-month-old rats an additional catheter was placed in the right carotid artery for intraarterial injections. All catheters were led subcutaneously to exit dorsally from a point between the scapulas.

Experimental Protocol

On the day of the experiment, individual conscious rats were placed in a cage measuring 30 x 30 x 35 cm. All experiments were conducted on conscious freely moving rats. The flow probes were connected via a spring-guarded cable from the head plug to a pulsed Doppler flow instrument constructed by the University of Iowa Bioengineering Resource Facility. Changes in blood flow velocity, measured as the Doppler shift in kHz, were recorded on a Beckman RM recorder. These changes in flow velocity have been documented to be directly and linearly proportional to volume flow. Electronically derived mean arterial pressure (MAP) was recorded from an Altech MS-20D low displacement pressure transducer connected to the intra-aortic cannula. Heart rate was recorded from the arterial pressure pulse using a Beckman 9857B tachometer. Experiments were begun after 1 hour or longer of acclimation.

Responses to 0.1 ml intravenous injections of 1.8 x 10^-6, 5.9 x 10^-6, 1.8 x 10^-5, 5.9 x 10^-5 moles/kg (0.03, 0.1, 0.3, and 1.0 μg/kg) norepinephrine and 9.7 x 10^-13, 2.9 x 10^-11, and 9.7 x 10^-11 moles/kg (0.01, 0.03, and 0.1 μg/kg) angiotensin II were obtained both before and after ganglionic blockade. In the 3-month-old rats, the same doses of pressor agents were also administered intraarterially. Sufficient time was allowed between injections for the recorded parameters to return to control levels. Ganglionic blockade was produced by the slow intravenous administration of 8.28 x 10^-4 moles/kg (30 mg/kg) hexamethonium bromide. To assess whether hexamethonium had actually interrupted all sympathetic transmission controlling the cardiovascular system, another group of 13-week-old SHR and WKY was implanted with chronic catheters, and arterial pressure responses to 1.15 x 10^-4 moles/kg (0.8 mg/kg) atropine sulfate and 2.65 x 10^-4 moles/kg (10 mg/kg) phentolamine mesylate were determined immediately after the administration of 8.28 x 10^-4 moles/kg (30 mg/kg) hexamethonium bromide. Atropine was used to evaluate the possibility that hexamethonium might have spared muscarinic ganglionic transmission, while phentolamine was then used to estimate residual neurogenic tone.

Responses of the vascular beds are expressed as percentage changes of vascular resistance from the control level. This is an accurate determination of changes in vascular resistance for the following reasons. Zero flow can be accurately measured by determining baseline with the ultrasound signal turned off (no Doppler shift occurs). Since the Doppler shift is directly proportional to volume flow, percentage changes in the shift are equivalent to the true percentage change in flow. The percentage change in resistance is then calculated ( Δ pressure/Δ% change Doppler shift).

Materials

The following drugs were used in this study: norepinephrine bitartrate (1-arterenol, Sigma), angiotensin II (Ciba), hexamethonium bromide (Sigma), atropine sulfate (Elkins-Sinn), and phentolamine mesylate (Regitine, Ciba).

Statistical Analysis

Group comparisons between SHR and WKY were performed using Student's t test. Data are expressed as means ± standard error. Comparison of dose responses to vasoconstrictor agents were carried out using an unweighted means solution of a two-factor
model with repeated measures on one factor. All data points of each of the pressor and vascular resistance responses to norepinephrine and angiotensin II were analyzed using the general linear means procedure of the statistical analysis system computer program. This procedure is designed to analyze unbalanced data. The model estimated the variation due to groups, animals within groups, log dose, and interaction between groups and log dose. The R-square for the overall model was also calculated for each set of responses.

Results
Response to Ganglionic Blockade

The contribution of neurogenic tone to vascular resistance and arterial pressure of conscious SHR and WKY controls was estimated by interrupting sympathetic transmission with hexamethonium. An example of the response to intravenous hexamethonium bromide is shown in figure 1. Ganglionic blockade reduced arterial pressure and vascular resistance in renal, mesenteric, and hindquarter beds of both SHR and WKY at 8 weeks of age, as shown in figure 2. Although the percentage and absolute changes in arterial pressure were similar in SHR and WKY, the arterial pressure of SHR was not significantly greater than WKY following hexamethonium. The decreases of vascular resistance in all three vascular beds were equivalent in SHR and WKY. Heart rate was reduced from 444 ± 21 to 403 ± 9 beats/min in SHR and from 414 ± 16 to 402 ± 12 beats/min in WKY.

The decreases in MAP and vascular resistance several minutes following ganglionic blockade in adult SHR and WKY are shown in figure 3. The MAP and vascular resistances of SHR were reduced by interruption of sympathetic transmission to an extent similar to that observed in WKY, and the arterial BP of SHR following hexamethonium remained significantly greater than similarly treated WKY. The immediate peak reduction of arterial BP was greater but still similar in SHR and WKY, −46 ± 2% and −48 ± 2% respectively. The reduction of vascular resistance was uniform in the renal, mesenteric, and hindquarter regions. Following hexamethonium, the heart rate of SHR fell from 423 ± 14 to 357 ± beats/min and the heart rate of WKY was reduced from 404 ± 18 to 352 ± 12 beats/min.

Response to Atropine and Phentolamine After Ganglionic Blockade

In another group of 13-week-old SHR (n = 8) and WKY (n = 8), hexamethonium reduced the MAP from 141 ± 3 to 98 ± 4 mm Hg in SHR (−30% ± 2%) and from 123 ± 4 to 79 ± 3 mm Hg in WKY (−36% ± 2%). To assess whether muscarinic receptors in the ganglia might make a significant contribution to pressor pathways, atropine was administered. Pressure was not changed significantly, averaging 95 ± 4 mm Hg in SHR and 83 ± 4 mm Hg in WKY. Phentolamine was then administered to assess whether arterial pressure was maintained by sympathetic transmission through ganglia resistant to blockade by hexamethonium and atropine. Phentolamine reduced the MAP of the SHR to 88 ± 5 mm Hg (−9% ± 3%) and of the WKY to 71 ± 8 mm Hg (−8% ± 8%). After each of these interventions, the arterial pressure of SHR remained significantly higher than that of WKY.

Response to Vasoconstrictor Agents

Vascular reactivity to graded doses of NE and angiotensin II was evaluated in conscious rats before and after ganglionic blockade. Responses to NE and angiotensin II before and after hexamethonium were not altered in SHR relative to WKY at 8 weeks of age. The arterial pressure and vascular resistance responses after hexamethonium to norepinephrine and to angiotensin II are summarized in figures 4 and 5. The heart rate response to 1 μg/kg NE was −15 ± 8 and −38 ± 12 beats/min in SHR and WKY respectively before hexamethonium. After hexamethonium, reflex bradycardia was abolished and the heart rate response to the same NE dose was 6 ± 11 and 6 ± 2 beats/min in SHR and WKY respectively.
In adult rats pressor and vascular resistance responses to intravenous NE were similar in SHR and WKY before hexamethonium (fig. 6). After ganglionic blockade, arterial pressure and mesenteric vascular resistance responses to NE were greater in SHR than WKY, while the renal vascular responses of SHR were smaller (fig. 7). Following hexamethonium, the pressor and vascular resistance responses to NE were greatly enhanced (cf. figs. 6 and 7). The change in heart rate elicited by 1 μg/kg intravenous norepinephrine was $-23 \pm 11$ beats/min in SHR and $-50 \pm 17$ beats/min in WKY before hexamethionium. After ganglionic blockade, the same NE dose reversed the heart rate response, increasing the rate in SHR and WKY by $33 \pm 10$ and $4 \pm 7$ beats/min respectively. Responses to intraarterial NE before ganglionic blockade were similar in SHR and WKY. The response of the mesenteric bed to intraarterial NE was greater in SHR than WKY in the presence of ganglionic blockade, but other responses were similar in the two groups.

Both before and after hexamethionium pressor and vascular resistance responses to intravenous and intraarterial angiotensin II in conscious SHR were not significantly different from those in WKY. The responses to intravenous angiotensin II following hexamethionium in 3-month old SHR and WKY are shown in figure 8.

**Figure 2.** Cardiovascular responses of seven conscious 8-week-old spontaneously hypertensive rats (SHR) (hatched bars) and six Wistar-Kyoto rats (WKY) (open bars) to hexamethonium bromide (HEX), $8.28 \times 10^{-4}$ moles/kg (30 mg/kg). Each bar represents the mean value, and the bracket above each bar represents the standard error. Mean arterial pressures before and after HEX are shown in the upper left, with the percent change given below the bars of both groups. The percent changes of vascular resistance following HEX are shown for the renal, hindquarter, and mesenteric vascular beds. Asterisks mark values that are significantly different from WKY ($p < 0.05$).

**Figure 3.** Cardiovascular responses of five conscious adult spontaneously hypertensive rats (SHR) (hatched bars) and six Wistar-Kyoto rats (WKY) (open bars) to hexamethonium bromide (HEX), $8.28 \times 10^{-4}$ moles/kg (30 mg/kg). Each bar represents the mean value, and the bracket above it represents the standard error. Mean arterial pressures before and after HEX are shown in the upper left, with the percent change given below the bars of both groups. The percent changes of vascular resistance following HEX are shown for the renal, hindquarters, and mesenteric vascular beds. Asterisks mark values that are significantly different from WKY ($p < 0.05$).
Discussion

The BP and vascular resistance of normotensive rats is maintained in part by neurogenic tone. This neural contribution could be estimated by interrupting sympathetic transmission, for example with a ganglionic blocking agent. If the BP of a hypertensive animal were due solely to sympathetic hyperactivity, ganglionic blockade should cause a larger percentage decrease of BP and vascular resistance to levels equivalent to those found in normotensive animals after ganglionic blockade. If the hypertension were not due exclusively to neurogenic factors, ganglionic blockade would not be expected to produce as great a fall of BP and vascular resistance, i.e., the final BP and vascular resistances would remain significantly elevated.

We recently tested this hypothesis in rats made hypertensive by baroreceptor deafferentation. Following ganglionic blockade, arterial pressure was reduced by a greater percentage in conscious hypertensive rats compared to their sham-operated controls and the post-blockade pressures were identical. Additionally, the percentage falls in vascular resistance in the renal and hindquarter beds were significantly greater in the hypertensive rats. Thus in a model of "pure" neurogenic hypertension, the experimental approach and instrumentation allowed us to unmask exaggerated neurogenic constrictor tone.

In distinction to rats with neurogenic hypertension, interruption of sympathetic nervous system transmission with hexamethonium failed to demonstrate elevated neurogenic vasoconstrictor tone in SHR.
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Figure 5. Responses to intravenous doses of angiotensin II following hexamethonium in seven conscious 8-week-old spontaneously hypertensive rats (SHR) (open circles and broken lines) and six Wister-Kyoto rats (WKY) (filled circles and solid lines). Plotted points represent the mean responses for each group at each dose administered. The plotted lines represent the least squares best fit linear regression to the responses of each group. The R-square values for pressure, renal resistance, hindquarter resistance, and mesenteric resistance responses are 0.8700, 0.7068, 0.8391, and 0.6541 respectively.

Relative to WKY. The changes in MAP and in renal, mesenteric, and hindquarter vascular resistances produced by hexamethonium were equivalent between SHR and WKY. The arterial pressure of conscious adult SHR remained significantly higher than the WKY following hexamethonium. These data suggest that neurogenic vasoconstrictor tone is not elevated in SHR, and arterial pressure and vascular resistance of the SHR are sustained at elevated levels by some other mechanism. These results are not in agreement with previous reports that sympathetic blockade caused a greater decrease in the arterial pressure of anesthetized SHR. 36-37 The discrepancy is perhaps explained by the presence of barbiturate anesthesia, an explanation that is supported by a number of other studies in conscious and chloralose anesthetized adult SHR which found that removal of the sympathetic nervous system by a variety of pharmacological and surgical interventions left arterial pressure of the SHR significantly higher than that of WKY. 16-18, 22, 23 In another study, arterial pressure responses to hexamethonium were very similar to ours in conscious young and adult SHR. 49

Failure to detect a major neural contribution in adult SHR could well mean that secondary structural and humoral mechanisms are more important to the maintenance of hypertension in mature SHR. To evaluate the possibility that neural mechanisms might be more significant in the development of hypertension in the young rat, identical studies were performed in 8-week-old SHR and WKY. The arterial pressure of 8-week-old SHR, although not yet at adult hyper-
Hypertension levels, was significantly higher than age-matched WKY. Administration of hexamethonium caused an equivalent drop in pressure and vascular resistance in the young SHR and WKY; however, the difference in arterial pressure between the SHR and WKY following hexamethonium was not significant. In the young SHR, the sympathetic nervous system activity may be partly responsible for the slightly elevated arterial pressure, perhaps by producing a higher neural contribution to heart rate. The absence of any differences in the vascular resistance changes suggests that the young SHR possess no greater neurally derived vasoconstrictor tone than young WKY. Although not found to be statistically significant, neural tone in the kidney of young SHR appeared to be slightly higher on the average. Since renal denervation in weanling SHR delays hypertension, the contribution of renal sympathetic mechanisms to spontaneous hypertension needs to be evaluated further.

These conclusions would not be justified if ganglionic blockade was not complete and the SHR were more resistant to the actions of hexamethonium than the WKY such that residual sympathetic vasoconstrictor tone was higher in the SHR than WKY. We have evidence that ganglionic block was complete in both SHR and WKY. Heart rate was significantly reduced in these animals. The baroreflex bradycardia in response to pressor agents was abolished. In 10 rats, additional doses of hexamethonium up to a total of 40 to 55 mg/kg failed to cause a greater reduction of blood pressure or vascular
responses to pressor agents were abolished. For example, before hexamethonium, i.v. norepinephrine causes only a modest increase in hindquarter vascular resistance due to baroreceptor mediated reflex vasodilatation that opposes the direct vasoconstrictor effect. After hexamethonium, the increase in hindquarter resistance was greatly magnified (cf. figs. 6 and 7). Hemodynamic responses produced by central nervous system stimulation in conscious rats are abolished by this dose of hexamethonium. Furthermore, administration of atropine and phentolamine in the presence of hexamethonium caused a similar percentage fall in BP of the two groups and failed to reduce the MAP of SHR to the level of similarly treated WKY. Phentolamine, an α-adrenergic receptor blocker, abolishes sympathetic vasoconstrictor tone by a different mechanism than ganglionic blockade and should have unmasked a neural component in SHR that might have been resistant to ganglionic blockade. The agent had no greater effect in SHR, thus sympathetic pressor pathways not blocked by hexamethonium do not seem to be responsible for the elevated pressure in 13-week-old SHR.

Physical factors could mask the presence of neurogenic factors in SHR. The arterial walls of SHR are thicker than those of WKY. Under the conditions of this study, arterial pressure was decreased by hexamethonium; thus, the distending pressure on the blood vessels is reduced leading to passive constriction which would oppose the dilation produced by removal of active neurogenic vasoconstrictor tone. If
the passive constriction in response to a lower distending pressure were greater in SHR than in WKY, the contribution of neurally-derived vasoconstrictor tone would be underestimated. We hope in future experiments to eliminate the complication of decreased distending pressure by selective lower spinal blockade of sympathetic transmission.

Several findings suggest, however, that the proposed role of passive vasoconstriction does not account for the present results. Changes in resistance produced by hexamethonium in young SHR and WKY were equivalent despite the fact that hypertrophic structural changes were unlikely to be as profound in young hypertensive rats compared to adults. Second, with the use of pressure-flow curves in perfused hindquarters, it was found that the contribution of neurogenic vasoconstrictor tone, estimated by cutting the sympathetic nerves, was the same in SHR and WKY. Since the effect of denervation was equivalent over the entire pressure range, passive vasoconstriction did not appear to mask high neurogenic vasoconstrictor tone in SHR.

Compensatory vasoconstriction in response to the reduced arterial pressure could also obscure the apparent neurogenic component of vasoconstrictor tone. When arterial pressure falls as the result of removal of the sympathetic nervous system, activation of humoral pressor systems would be expected. Immediately following the intravenous infusion of hexamethonium, pressure and vascular resistance fell to a maximum and then rose gradually to a plateau several minutes later. This plateau level was the post-gangli-
No attempt was made to measure cardiac output or total peripheral resistance in the present study. Previous work has indicated that cardiac output of SHR is similar to WKY. The increase in arterial pressure appears to be due to a uniform increase in peripheral vascular resistance with no redistribution of blood flow. Neither we nor others have measured the change in cardiac output following blockade of the sympathetic nervous system of the SHR. We did find uniform decreases in vascular resistances in the renal, hindquarter, and mesenteric regions within both the SHR and the WKY as well as in the same beds between strains. Thus, the three regions we have studied appear to demonstrate roughly equivalent neurogenic vasoconstrictor tone in the resting conscious state.

If vascular responsiveness to the sympathetic transmitter NE were abnormally low, the present findings could be interpreted as evidence for high sympathetic nervous discharge which is not functionally converted to high vasoconstrictor tone. Thus, it was important to evaluate vascular responsiveness under the same conditions. Both increases and decreases in vascular reactivity of SHR relative to WKY have been reported, depending upon the vascular region, and age of the rats. In the present study, reactivity to angiotensin II and norepinephrine in the renal, hindquarter, and mesenteric vascular beds were similar in 8-week-old SHR and WKY. In adult SHR after ganglion blockade, the pressor and mesenteric vascular resistance responses to NE were increased, and renal vascular resistance responses to NE were decreased. These changes in reactivity of 3-month-old SHR are apparently fairly specific, since angiotensin II responsiveness in these same animals was similar to WKY. These data argue against a non-specific structural change leading to increased vascular reactivity to all vasoconstrictors.

The increased responsiveness to NE in the renal vascular region confirms an earlier report from this laboratory. This characteristic of the renal vessels could reconcile previous reports of increased renal sympathetic nerve activity with the present demonstration of normal renal neurogenic vasoconstrictor tone. Renal vasoconstrictor tone could be normal if more transmitter were released by the higher nerve activity, but vascular smooth muscle response was depressed. The results of the present study do not confirm previous reports, several of which are from this laboratory, of increased vascular reactivity to pressor agents in isolated perfused kidneys and hindquarters. It may well be that factors such as anesthesia, perfusion pumps, and artificial perfusion media have a more profound effect on vascular reactivity than has been appreciated. It is also possible that increased vascular reactivity to norepinephrine might be present in conscious SHR prior to 8 weeks of age.

The present study, unlike any previous investigations, has assessed directly the component of vascular resistance due to neurogenic factors in conscious SHR versus age-matched WKY. Under these conditions, neurogenic vasoconstrictor tone appears not to be the major factor responsible for the increased arterial pressure and vascular resistance in SHR. It should be emphasized that these data do not necessarily conflict with the numerous studies that provide either direct or indirect evidence for exaggerated sympathetic activity in SHR. Such altered activity, if it were associated for example, with a defect in the nerve terminal release of norepinephrine, could fail to be converted to the crucial functional equivalent, high vascular smooth muscle tone of neurogenic origin.

References

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