Association Between Sympathetic Nerve Activity and Cerebrovascular Protection in Young Spontaneously Hypertensive Rats

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SUMMARY The purpose of this study was to determine resting and maximal superior cervical sympathetic nerve activity in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) at five and ten weeks of age as hypertension was developing. Basal cervical sympathetic nerve activity (SNA) of five week SHR was 58 ± 3 \( \mu V \) which was significantly elevated over age-matched WKY (SNA = 30 ± 4 \( \mu V \), *p < 0.001) and ten week SHR (SNA = 30 ± 4 \( \mu V \), *p < 0.001) as well as ten week WKY (SNA = 24 ± 4 \( \mu V \), *p < 0.001). Thus, during basal conditions five week SHR nerve traffic was approximately two times that found in age-matched WKY as well as in ten week SHR and WKY. The peak sympathetic nerve activity in response to rapid hemorrhage in five week SHR (215 ± 16 \( \mu V \)) was significantly elevated over the maximal response of WKY (140 ± 23 \( \mu V \)) (*p < 0.02). Ten week SHR also reached a maximal sympathetic nerve activity (187 ± 28 \( \mu V \)) that was significantly elevated over WKY (100 ± 15 \( \mu V \)) (*p < 0.02). Thus, both five and ten week SHR had a greater capacity for elevated nerve activity following rapid hemorrhage than age-matched WKY. The elevation in cervical sympathetic activity in five week SHR, and the elevated capacity for sympathetic neural response in both five as well as ten week SHR, are consistent with a central nervous system abnormality in SHR that could relate to the previously described protective influence of sympathetic nerves on SHR cerebral blood vessels as hypertension is developing.

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THE PRESENCE OF SYMPATHETIC NERVES has been associated with a long-term influence on blood vessels. During growth, sympathetic nerves have been reported to contribute to smooth muscle mass and vascular resistance. In the spontaneously hypertensive rat (SHR), a decrease in cerebrovascular wall/lumen ratio and enhanced blood-brain barrier permeability have been reported after early superior cervical ganglionectomy prior to the development of hypertension. These data suggest that the absence of the chronic influence of sympathetic nerves on the cerebrovascular bed early during hypertension lead to a decreased wall thickness and greater blood-brain barrier (BBB) permeability under certain conditions. A similar protective effect of sympathetic nerves on the brain vasculature has not been observed in older SHR after the development of hypertension (Mueller and Ertel, unpublished data). Thus, the long-term protective influence of sympathetic nerves on SHR cerebrovascular properties appears to be present only in younger animals prior to the marked development of hypertension.

Elevated sympathetic nerve activity has been reported in SHR compared to the normotensive Wistar-Kyoto (WKY). Since WKY do not demonstrate the same long-term influence of sympathetic nerves on brain vasculature found in SHR, we reasoned that elevated sympathetic nerve activity in SHR could relate to the chronic influence observed. Thus, the purpose of this study was to determine resting and maximal sympathetic nerve activity in SHR and WKY at two different ages: at five weeks prior to the development of marked hypertension and at ten weeks after hypertension was well developed. In this way the relationship of sympathetic nerve activity to the protective influence of sympathetic nerves could be evaluated as well as a possible age-related central sympathetic neural defect.

Methods We used five and ten week male SHR and WKY (Taconic Farms). Data from eight SHR and seven WKY was used in the five week group; data from eight SHR and nine WKY was used in the ten week group. All rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.). A tracheal cannula was inserted to facilitate spontaneous respiration via a midline cervical incision. The right carotid artery was catheterized with thin-walled PE 50 tubing filled with heparinized saline for measurement of blood pressure. The left superior cervical trunk proximal to the superior cervical ganglion was separated from the vagus and aortic depressor nerves to record sympathetic nerve activity from it. The sympathetic nerve fibers were identified and stripped of their connective tissue coverings using 20X to 40X magnification (Karl Zeiss, model 64051).

Recordings from multifiber preparations were made by placing the nerves on bipolar stainless steel electrodes. The nerves and electrodes were bathed in a pool of mineral oil to prevent drying. The nerve signals were detected with an AC differential preamplifier (Grass Instruments, model P-15) with a time constant of three milliseconds. The amplified nerve signals were displayed on a Tektronix oscilloscope (model 5103) for visualization and photographing with a Tektronix. 

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tronix camera (model C-12). These signals were further amplified using a high gain operational amplifier, then rectified using a full wave rectifier circuit and integrated continuously with a RC integrator (time constant = 20 milliseconds). This integration method and its limitations have been described by Ninomiya et al. The integrated signals were averaged using a RC network with a time constant of one second. Therefore, the nerve data presented in this paper are expressed as mean superior cervical sympathetic nerve activity (SNA) and is calibrated in microvolts (µV) above noise level. The noise level was determined in each experiment by shortening the input electrodes and this value was electronically subtracted from the electromyogram by adjusting the zero bias level on the recording system. All signals — the blood pressure, superior cervical sympathetic nerve activity and the electrocardiogram — were recorded on a Beckman Type R Dynograph.

Within 45 minutes to one hour after sedation, cervical sympathetic nerve activity was recorded during resting conditions for 15 minutes in all animals. The average sympathetic nervous activity during that period was determined and designated as resting sympathetic nerve activity. In some animals (three SHR and three WKY) nerve activity was recorded for 45 minutes to determine if minor differences in the timing of nerve traffic after sedation would alter our results.

In order to obtain a maximal sympathetic discharge, the animals were sacrificed by allowing them to hemorrhage rapidly which produced central ischemia. A midline incision was made in the upper abdomen, the aorta was isolated and then partially severed. Rapid hemorrhage over less than five seconds followed (fig. 3). Those animals that failed to demonstrate a substantial increase in nerve traffic (> 50 µV) (fig. 1) during this strong sympathetic stimulus were excluded from the data analysis for the study because of the possibility of nerve damage. Cervical sympathetic nerve activity was recorded continuously after hemorrhage until it fell below the basal level (up to 15 minutes).

Results

Resting Sympathetic Nerve Activity

The mean arterial pressure of the five week SHR was significantly elevated over the age-matched WKY [SHR 113 ± 6*, WKY 78 ± 6 mm Hg (mean ± se) *p < 0.001] during resting conditions. Likewise the mean arterial pressure of the ten week SHR was significantly elevated over the age-matched WKY [SHR 173 ± 8*, WKY 109 ± 2 (mean ± se) *p < 0.001]. The difference between the SHR and WKY mean arterial pressure almost doubled between five and ten weeks of age. Resting sympathetic nerve activity of five and ten week WKY and SHR is shown in figure 2. Basal cervical sympathetic nerve activity of five week SHR was 58 ± 3 µV* which was significantly elevated over age-matched WKY (SNA = 30 ± 4 µV, *p < 0.001) and ten week SHR (SNA = 30 ± 4 µV, *p < 0.001) as well as ten week WKY (SNA = 24 ± 4 µV, *p < 0.001). Ten week SHR nerve traffic (30 ± 4 µV) was elevated over five week WKY (24 ± 4 µV) but the difference was not significant. Thus, during basal conditions five week SHR nerve traffic was approximately two times that found in age-matched WKY and in the ten week SHR and WKY. At ten weeks of age, SHR nerve traffic was elevated over age-matched WKY, but the difference was not significant.

Maximal Sympathetic Nerve Activity

The peak sympathetic nerve activity in response to rapid hemorrhage in five and ten week SHR and WKY is shown in figure 3. The average time to peak sympathetic nerve activity was 104 ± 5 seconds. Five week SHR reached a maximal sympathetic nerve activity (215 ± 16 µV*) that was significantly elevated over the maximal response of the age-matched WKY (140 ± 23 µV) (*p < 0.02). Ten week SHR also reached a maximal sympathetic nerve activity (187 ± 28 µV*) that was significantly elevated over the age-matched WKY (100 ± 15 µV) (*p < 0.02). Thus, both five and ten week SHR had a greater capacity for elevated nerve activity during central ischemia than age-matched WKY. However, the peak sympathetic nerve activity of ten week SHR was significantly elevated over five week WKY (p < 0.001) as well as ten week SHR (p < 0.001) and WKY (p < 0.001).

![Figure 1](image1.png)

**Figure 1.** Mean arterial pressure (MAP) and cervical sympathetic nerve activity (SNA) in nine week spontaneously hypertensive rats (SHR) before and after rapid hemorrhage which led to central ischemia. After the MAP falls rapidly to 0, SNA increases to a maximal response in about 100 seconds.

![Figure 2](image2.png)

**Figure 2.** Resting cervical sympathetic nerve activity (SNA) in five and ten week spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY). The SNA activity of five week SHR is significantly elevated over five week WKY (p < 0.001) as well as ten week SHR (p < 0.001) and WKY (p < 0.001).
activity of five week WKY (140 ± 23 μV) did not differ from ten week WKY (100 ± 15 μV) (p < 0.20) nor did the peak sympathetic nerve activity of five week SHR (215 ± 16 μV) differ from ten week SHR (187 ± 28 μV) (p < 0.50).

The increase in nerve traffic after rapid hemorrhage was similar in both five and ten week SHR (fig. 4). In five week SHR nerve traffic increased 156 ± 15 μV (58 ± 3 to 215 ± 16 μV) while ten week SHR increased 157 ± 27 μV (30 ± 4 to 187 ± 28 μV). Likewise, there was not a difference between the increase in five week WKY nerve traffic {109 ± 25 μV (30 ± 4 to 140 ± 23 μV)} compared to ten week {77 ± 13 μV (24 ± 4 to 100 ± 15 μV)}. Thus, both five and ten week SHR increased in nerve activity essentially the same (156 ± 15 and 157 ± 27 respectively)

Discussion

This study indicates that resting superior cervical sympathetic nerve activity is elevated in five week SHR compared to age-matched WKY and ten week SHR plus WKY. In addition, the capacity for elevated maximal sympathetic nerve activity was present in both the five and ten week SHR compared to WKY. The elevation in resting cervical sympathetic activity in five week SHR, and the elevated capacity for sympathetic neural response in both five as well as ten week SHR, are consistent with a central nervous system abnormality in SHR that could relate to the protective influence of sympathetic nerves on SHR cerebral blood vessels.

There are several aspects of this design that should be examined. We measured sympathetic nerve activity in lightly anesthetized rats using standard techniques. Since the level of anesthesia could influence nerve traffic,9 we used uniform concentrations of sodium pentobarbital/weight and measured nerve traffic over a prolonged time period in several SHR and WKY. We reasoned that an elevated degradation of anesthetic in SHR might falsely elevate sympathetic nerve traffic because of lighter sedation of SHR compared to WKY. We found that when we measured sympathetic nerve traffic in SHR and WKY over a prolonged period (up to 45 minutes) nerve traffic remained constant. Therefore, we do not believe that the level of sedation of SHR compared to WKY influenced our results.

Another consideration in the design of this study was the evaluation of maximal sympathetic activity in addition to resting sympathetic nerve activity. This additional measurement offered an opportunity to judge the physiologic status of our preparation. Rapid hemorrhage, used to produce maximal sympathetic nerve activity, is a strong stimulus since it produces brain ischemia.10 A maximal sympathetic response to this stimulus is produced in one–two minutes.11 This known time course correlated with the time necessary to produce the profound increase in nerve activity that we noted in this study (104 ± 5 seconds). The capacity of the sympathetic central and efferent limb to lead to profound peripheral vasoconstriction, elevated blood pressure and perfusion of the brainstem is tested by this system.10 Animals that did not respond to central ischemia with an elevation in nerve traffic had probable nerve damage and were excluded from our study. If the resting nerve activity found in SHR at five weeks of age had been elevated for extraneous reasons, the capacity for an increase in nerve activity during central ischemia would have been expected to be compromised. This was not the case. During rapid hemorrhage, five week SHR nerve traffic increased to the
same degree as ten week SHR (156 ± 15 versus 157 ± 27 respectively).

There is considerable evidence that supports an involvement of central catecholaminergic mechanisms in SHR hypertension and that the changes may be age-related. Haeusler et al.12,13 showed that intraventricular 6-hydroxydopamine administration to adult SHR had no effect on blood pressure; however, similar treatment in young SHR did markedly attenuate the rise in blood pressure. Le Quan-Bui et al.14 demonstrated reduced norepinephrine content in some medullary and hypothalamic nuclei in SHR at four but not 12 weeks of age. More recently, Morris et al.15 have described a selective increase in the concentration of hypothalamic adrenoreceptors in four-week old SHR prior to the development of hypertension. Thus, a central sympathetic abnormality appears to be present in SHR, and this study plus others12-15 suggests that the defect may be more pronounced early in the development of hypertension.

In addition to our previous observation that the long-term effect of sympathetic nerves is age-related16 and protects the BBB under certain conditions,1 Sadoshima and colleagues16 have reported an age-related protection of sympathetic nerves against stroke. These investigators found that unilateral superior cervical ganglionectomy of stroke-prone SHR at an early age lead to a significantly increased incidence of stroke (hemorrhagic plus ischemic) on the denervated side. However, when superior cervical ganglionectomy was performed in older animals, no predisposition to stroke on the denervated side was observed. Thus, the influence of sympathetic nerves on the brain vasculature again was present in the young animal prior to the development of hypertension but not in the older animal after an elevated blood pressure had been established.

Could this elevated resting sympathetic nerve activity in five week SHR contribute to structural vascular alterations,4 the protection of the BBB5 and the protection against stroke16 previously observed? We can conjecture two possibilities. One is that the elevated sympathetic tone in the young may act acutely by increasing precapillary resistance during blood pressure elevation and lead to decreased capillary pressure, thus protecting against vascular damage. Another possibility is that the chronic effect of exaggerated fluctuations in an already elevated resting sympathetic nerve activity may lead to changes in vascular tone and “exercise” of smooth muscle which has been postulated to result in vascular hypertrophy17 that is protective.18

There are several lines of evidence from human studies which indicate that essential hypertension in the young may be neurogenic in origin similar to the SHR, the animal model of human essential hypertension. Sever et al.19 have demonstrated differences in levels of plasma norepinephrine in hypertensive versus control subjects in response to postural change that were more pronounced in younger subjects. In addition, Falkner et al.20 have demonstrated a sustained increase in systolic and diastolic blood pressure and heart rate during stress in labile adolescent hypertensive compared to the normotensive controls. Even those adolescents with normal blood pressure but one hypertensive parent showed a significant difference in diastolic pressure and heart rate during stress than the controls. The post-stress plasma catecholamines were also higher in the labile hypertensive and genetically hypertensive groups than in the controls. As a group, these findings demonstrate increased central nervous system mediated adrenergic activity in young individuals that are genetically hypertensive.

In conclusion, the elevated resting sympathetic nerve activity observed in five week SHR may contribute to protection against BBB damage and/or stroke during the development of hypertension: 1) acutely by elevating precapillary vascular resistance and 2) chronically by exerting a long term influence on blood vessels that enhances vascular hypertrophy and increases precapillary resistance. The origin of the elevated sympathetic nerve activity is most probably related to a central sympathetic nervous system abnormality that is also expressed by the elevated capacity for sympathetic outflow during central ischemia in both five and ten week SHR. We conjecture that these findings may also relate to human hypertension in which increased adrenergic participation has been reported in the young19.20

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