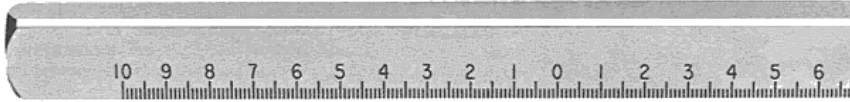




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**COMPARISON OF SYMPATHETIC REFLEX ACTIVITY ELICITED BY
SINGLE SHOCK TO A-TYPE AFFERENT SOMATIC FIBRES IN
NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS**

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Introduction

Recordings of multifiber nerve activity in spontaneously hypertensive rats (SHR) show increased central sympathetic discharge to various organs (4, 5, 8), the extent of which is related to blood pressure (6). The cause of the hyperactivity is still not clear. It is possible that a general increase in excitability of elements within the central nervous system (CNS) of SHR that regulate sympathetic activity is a main cause of the hyperactivity.

It is known that at least two neural mechanisms are responsible for maintenance of sympathetic drive to heart and vessels: sympathoexcitatory bulbospinal neurons located within the rostral ventrolateral medulla (15) and reflexes involving cranial and spinal afferent pathways (2). Specifically, the medullar neurons are excited by the posterior hypothalamus and brainstem neurons that originate from the activation of peripheral receptors (9). A hyperresponsiveness in the defense response system is suggested by the findings (3, 7) that environmental stress produces larger increases in blood pressure, heart rate, plasma catecholamine levels, and sympathetic nervous

activity in SHR than in the Wistar-Kuoto (WKY) rats, a normotensive genetic control strain. The hypothesis that somatosympathetic reflexes might be enhanced in SHR versus WKY rats is based upon indirect experimental observations. For example, electrophysiological properties of rostral ventrolateral medulla neurons in SHR differ from those found in normotensive rats and may contribute to the increased sympathetic outflow from rostral ventrolateral medulla in this strain (1). A recent study demonstrated that sciatic nerve stimulation elicited significantly greater increases in mean arterial pressure and in heart rate in SHR than in WKY rats (10). Sympathetic electrical activity was not recorded in this study. There are little data to establish a contribution of spinal systems in cardiovascular control and to the effect of sympathetic hyperresponsiveness in SHR in particular (13).

Therefore, the purpose of this study is to compare the reflex responses in preganglionic sympathetic nerves evoked by somatic afferent stimulation in SHR and in WKY rats and to investigate the relative importance of the spinal cord compared to the medullary brain in mediating the somatosympathetic reflex in hypertensive rats..

Methods

The experiments were performed using 5 adult male normotensive WKY rats and 5 adult male spontaneously hypertensive SHR rats, aged between 16 and 19 weeks. Anesthesia was initially induced with ether *pro narcosi*. Both femoral veins and one femoral artery were cannulated. The left veinose catheter was used for the administration of an initial dose of α -chloralose (70 mg/kg, Aldrich) and for maintenance doses (20-30 mg/kg/h) throughout the surgical preparation and experiment. The right veinose catheter was used for administration of drugs. Pipecurii bromidum (0.3 mg/kg/h, Gedeon Richter) was infused to avoid animal movements that might disturb stable recording of sympathetic nerve activity during stimulation of a somatic nerve. The rats were artificially ventilated with room air via tracheostomy. Femoral arterial pressure and heart rate were recorded using standard procedures. Core body temperature was measured with a thermistor probe inserted into the colon and was kept between 37.5-38.0°C by a temperature-controlled surgical table.

Sympathetic tonic multifiber electrical activity and somatosympathetic reflexes were recorded biphasically with a platinum hook bipolar electrode after capacity-coupled preamplification (band pass 10-3,000 Hz) from the central end of cut cervical sympathetic trunk. After amplification, the signals were monitored on an oscilloscope and recorded on a polygraph. The evoked somatosympathetic responses were averaged by a poststimulus signal-averaging program. The left cervical sympathetic trunk was isolated from a ventral approach 11.5 cm caudal to its enter in superior sympathetic ganglion. The median nerve was isolated from the surrounding tissue of the left forelimb and the central of the crushed nerve was placed on platinum bipolar hook electrode for stimulation and then covered with warm mineral oil. Electrical stimuli (5-10 V with 0.5 ms duration) were applied via an isolation unit.

Spinal transection in four spontaneously hypertensive rats was done following control recording of somatosympathetic reflexes. The sympathetic and somatic nerves were removed from electrodes. Monitoring of blood pressure and heart rate was continued. The rat was turned on its back. The first cervical vertebra and the occipital bone were exposed via a midline dorsal incision. The spinal cord between them was completely transected with a scalpel blade. Then an animal was returned in the initial position, and the nerves were replaced on the electrodes. Electrical stimulation was

applied every the thirtieth minute during 2 hours after the spinal transection. Quality of the transection was examined after the death of rat.

Results

The basal value of mean arterial pressure in the SHR rats (166 ± 7.6 mmHg) was significantly ($p<0.01$) higher than the basal value of mean arterial pressure in the WKY rats (122 ± 3.4 mmHg). The heart rate in the SHR (153 ± 6.0 ms) did not differ from the heart rate in WKY rats (146 ± 3.4 ms).

The single shock to somatic myelinated afferent fibres in normotensive rats elicited a response in sympathetic nerves that consisted of four discharges (fig. 1). Their characteristics are shown in table 1. Discharges II and III were the largest amplitude and sometimes they merged into one discharge. Discharges II, III and IV were evoked by threshold stimulation. Discharge I was of smallest amplitude and was elicited by the more intense stimulation. Our study showed that the discharge I is of spinal origin and the discharges III, IV were of supraspinal origin (14). Discharge II consisted of spinal and supraspinal reflex components.

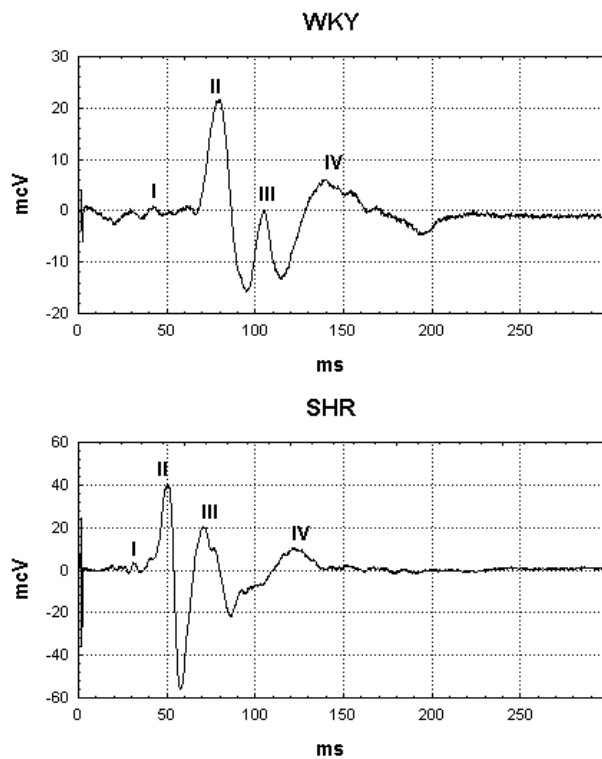


Fig. 1. Somatosympathetic reflex in the cervical sympathetic trunk in WKY rat and SHR. Reflexes illustrated have been signal-averaged from 40 responses to single-pulse stimulation of median nerve. Note the difference in scales between WKY and SHR.

In spontaneously hypertensive rats, single shock stimulation of A afferent fibres elicited a response in the cervical sympathetic trunk (fig. 1). Its shape was similar to that of somatosympathetic reflex in the Wistar-Kyoto rats. However, the amplitude of every discharge was higher and the latent period of every discharge was

shorter than the amplitude and latent period of the reflex discharge in the Wistar-Kyoto rats (table 1). Threshold stimulation of somatic nerve elicited discharge II. Electrical stimulation at two thresholds elicited the discharges II and III. The fourth discharge was evoked by a stimulation of 2-5 thresholds.

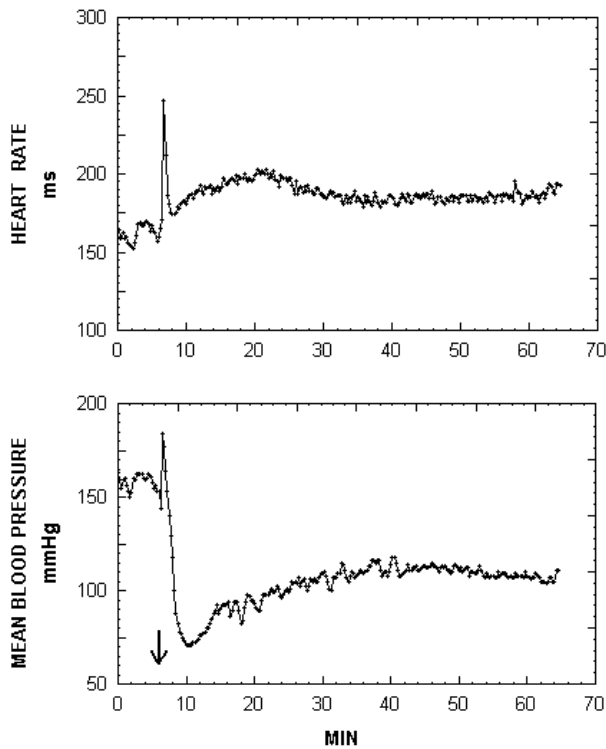


Fig. 2. Dynamics of heart rate, mean blood pressure in spontaneously hypertensive rat spinalized at the 1st cervical level. The arrow indicates the transection of the spinal cord.

In three hypertensive rats, the spinal transection resulted in a disturbance in control of circulation. Mean blood pressure and heart rate decreased to 70-80 mmHg and 190-220 ms, respectively. In 25-30 min after spinalization, mean blood pressure and heart rate stabilized at 90-115 mmHg and 178-195 ms, respectively (fig. 2). Tonic sympathetic activity was suppressed and 50-60 min later reappeared. For next 60-70 min, sympathetic activity increased to a level that was below the control level. The initial elevation of blood pressure was caused by mechanical stimulation of descending sympatho-exciting pathways in spinal cord during the spinal transection. A reflex response evoked by the same somatic nerve stimulation was not observed after spinalization (fig. 3) for some time. Ninety minutes after spinal transection, we again recorded reflex discharges, the latent period and duration of which conformed to discharge II in spontaneously hypertensive rats with an intact central nervous system. The amplitude of this spinal discharge was decreased, however. In 120 min after spinalization discharge 1 had reappeared. (fig. 3).

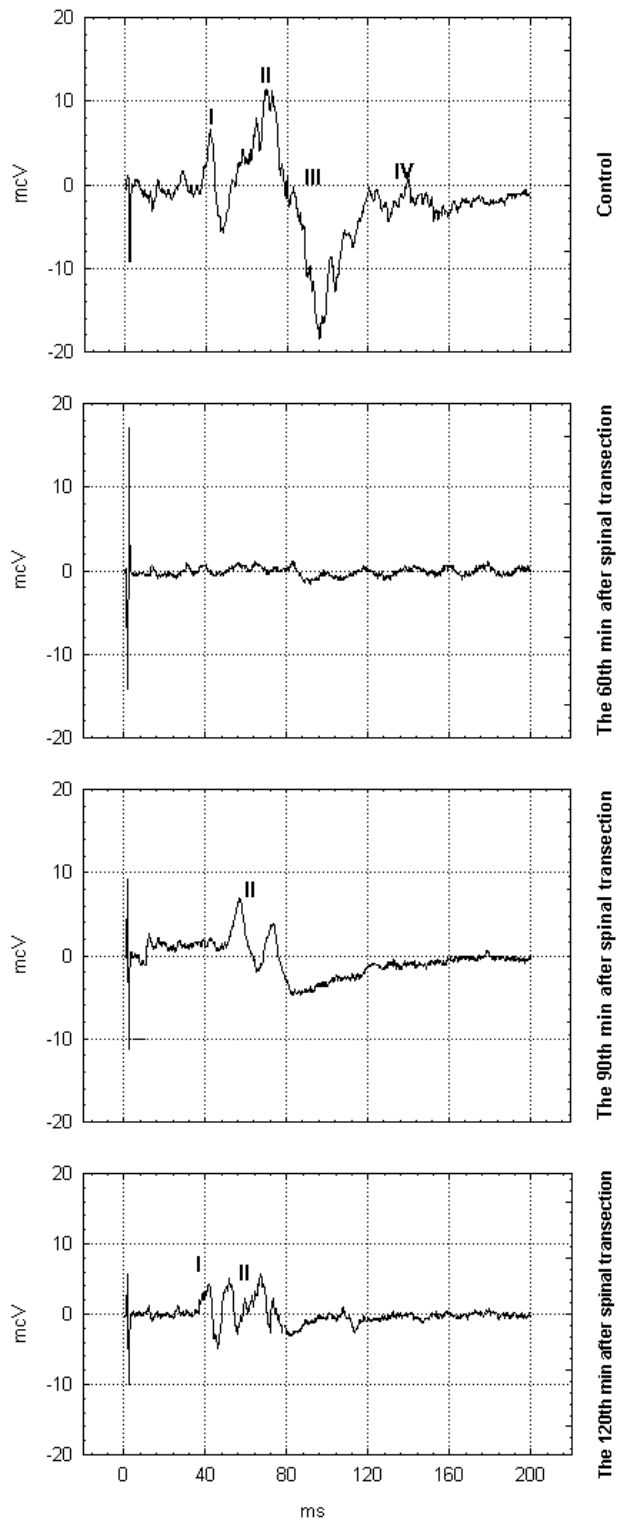


Fig. 3. Somatosympathetic reflex in spontaneously hypertensive rat spinalized at the 1st cervical level.

In one experience, the spinal transection resulted in the decrease of mean arterial pressure to 45 mmHg. The arterial pressure stabilized at 70-80 mmHg. Tonic and evoked sympathetic activity were suppressed for all the entire study.

Table 1. The characteristics of the somatosympathetic reflex in normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).

Characteristic	Strain	Reflex discharge			
		I	II	III	IV
Latent period (ms)	WKY	27.4±3.78	63.0±2.96	84.8±4.03	116.4±4.93
	SHR	32.9±1.48	44.9±2.68	73.2±3.33	112.1±4.03
Duration (ms)	WKY	6.3±0.68	15.7±2.08	12.1±2.64	41.6±6.64
	SHR	3.9±0.86	20.7±3.22	18.7±4.32	29.3±2.78
Amplitude (mV)	WKY	1.9±0.43	12.3±3.16	11.7±3.29	7.4±3.42
	SHR	3.9±0.84	25.4±3.42	15.9±5.30	8.3±2.47

Discussion

We limited specifically the intensity of electrical stimulation to subthreshold levels for nonmyelinated afferent fibres (C fibres) because it was difficult to record the C-reflex in a preganglionic nerve. In SHR in comparison with normotensive rats, discharge I was of higher amplitude but was of shorter duration. Recording of multifiber nerve response may show an increased burst if neurons are aroused more synchronously, for reduced time. Consequently, there was no reason to believe that discharge I in SHR was enhanced. Discharge IV was similar in both strains. In the SHR, the duration of the two biggest discharges was longer than that in the WKY rats. Therefore, we concluded that the somatosympathetic reflex in CNS intact, spontaneously hypertensive rats was more powerful than the somatosympathetic reflex in the normotensive rats.

Reflex power is a characteristic of reflex discharge (11). It is defined as the area under a discharge curve. A contribution of spinal cord sympathetic neurons in the reflex response was determined by a ratio of reflex power in spinalized rats to reflex power in CNS-intact rats. In spontaneously hypertensive rats this ratio was 24.6 %. In our preliminary investigation on Wistar rats, the power of spinal somatosympathetic reflex was 13.8 % (14). Thus, the responsiveness of sympathetic preganglionic neurons in spontaneously hypertensive rats is increased in comparison with normotensive Wistar rats.

The hyperactivity and hyperreactivity seen in the sympathetic nervous system of the SHR indicate a difference between strains in those pathways responsible for the background activity in sympathetic nerves and for the phasic increases in activity consequent to somatic afferent stimulation. This difference could arise from alterations in the number or activity of presynaptic elements in such pathways or from changes in the responsiveness of postsynaptic elements to these inputs. On the other hand, the hyperactivity and hyperreactivity in SHR may be caused by diminished inhibitory influences to medullospinal and spinal neurons (12).

In conclusion, our results provide new information on sympathetic nervous responsiveness to somatic afferent stimulation in SHR. It is suggested that the enhancement of sympathetic responses to peripheral stimuli may play an important role in the pathogenesis of hypertension.

References

1. Chan R. K. W., Chan Y. S., Wong T. M. Electro-physiological properties of neurons in the rostral ventrolateral medulla of normotensive and spontaneously hypertensive rats. *Brain Res.* 549: 118-126. 1991.
2. Coote J. H., Perez-Gonzalez J. T. Response of some sympathetic neurons to volley in various afferent nerves. *J. Physiol.*, 208(2): 261-278. 1970.
3. Hallback M., Folkow B. Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. *Acta Physiol. Scand.* 90: 684-698. 1974.
4. Iriuchijima J. Sympathetic discharge rate in spontaneously hypertensive rats. *Jpn. Heart. J.* 14: 350-356. 1973.
5. Judy W.V., Watanabe A.M., Henry D.P., Besch H.R., Murphy Jr.W.R., Hockel G.M. Sympathetic nerve activity. Role in regulation of blood pressure in spontaneously hypertensive rat. *Circ. Res.* 38: II21-II29. 1976.
6. Judy W.V., Watanabe A.M., Murphy Jr.W.R. et al. Sympathetic nerve activity and blood pressure in normotensive backcross rats genetically related to the spontaneously hypertensive rats. *Hypertension.* 1: 598-613. 1979.
7. Li S. G., Lawler J. E., Randall D. C., Brown D.R. Sympathetic nervous activity and arterial pressure responses during rest and acute behavioral stress in SHR versus WKY rats. *J. Auton. Nerv. Syst.* 62(3): 147-154. 1997b
8. Lundin S., Ricksten S.E., Thoren P. Renal sympathetic activity in spontaneously hypertensive rats and normotensive controls, as studied by three different methods. *Acta Physiol. Scand.* 120(2): 265-272. 1984.
9. Morrison S. F., Reis D. J. Reticulospinal vasomotor neurons in the RVL mediate the somatosympathetic reflex. *Am. J. Physiol.* 256: R1084-R1097. 1989.
10. Ruggeri P., Cogo C. E., Picchio V., Ermirio R., Mary D. A. S. G. Reflex cardiovascular responses to somatic stimulation in spontaneously hypertensive rats. *J. Hypertension.* 18: 595-600. 2000.
11. Sato A., Schmidt R. F. Somatosympathetic reflexes: afferent fibres, central pathways, discharge characteristics. *Physiol. Reviews*, 53(4): 916-947. 1973.
12. Schramm L. P., Barton G. N. Diminished sympathetic silent period in spontaneously hypertensive rats. *Am. J. Physiol.* 236(3): R147-R152. 1979.
13. Schramm L. P., Chornoboy E. S. Sympathetic activity in spontaneously hypertensive rats after spinal transection. *Am. J. Physiol.* 1982 Nov. 243(5): R506-R511.
14. Shcherbin Yu. I., Khrustaleva R. S., Tsyrlin V. A. A study of spontaneous and reflex electrical efferent activity in sympathetic nerve of rats. *Rus. J. Physiology.* 86(3): 320-328. 2000.
15. Spyer K. M. Central nervous mechanisms contributing to cardiovascular control. *J. Physiol.* 474(1): 1-19. 1994.