DUAL EFFECT OF SYMPATHETIC HYPERFUNCTION ON BLOOD VESSELS IN SPONTANEOUSLY HYPERTENSIVE AND STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY

• *The sympathetic nervous system has been considered to be hyperactive from the very beginning after birth in spontaneously hypertensive and stroke-prone spontaneously hypertensive rats. This is a primary factor for the development and maintenance of hypertension via structural and functional alterations of the arteries and the heart. It is. also described that the sympathetic hyperfunction probably play a protective role in necrosis of vascular smooth muscle cells in spontaneously hypertensive and stroke-prone spontaneously hypertensive rats.*

INTRODUCTION

The spontaneously hypertensive rats (SHR) were developed by Okamoto and Aoki (1) for elucidating the pathogenic factors of the human essential hypertension. Three stages were distinguished in relation to the blood pressure: prehypertensive stage (0-30 days after birth), developmental stage (40-90 days after birth) and the established stage (over 90 days after birth). Many biochemical investigations showed abnormal functions of both central and peripheral sympathetic nervous system in SHR and stroke-prone spontaneously hypertensive rats (SHRSP). A dysfunction of the nucleus loci coerulei (2), an increased basal sympathetic tone of the superior cervical

ganglia (SCO) (3) and the celiac ganglia (4), and an increased noradrenaline (NA) content in the serum (5, 6) and in various tissues (7-9) were described. These authors, however, reported the functional state of the sympathetic nervous system in SHR and SHRSP during the developmental and established stages of hypertension, but not during the prehypertensive stage.

In the following review we describe the morphological studies related to the functional state of the peripheral sympathetic nervous system of SHR and/or SHRSP throughout the prehypertensive to the established stages of hypertension and the effects of the hyperfunction of peripheral sympathetic nervous system on the cardiovascular and cerebrovascular systems.

FUNCTIONAL ACTIVITY OF SUPERIOR CERVICAL GANGLIA AND STELLATE GANGLIA OF SPONTANEOUSLY HYPERTENSIVE RATS

• **Ganglion weight, ganglion cell volume and ganglion cell area**

The peripheral sympathetic nervous system regulates blood pressure by modulating the peripheral resistance of the vessels. It has been thought to be one of the most prominent factors in the development of hypertension in SHR. There have been many morphological, physiological and biochemical studies on

the sympathetic nervous system of SHR after the onset of hypertension (10-14), but only a few studies were carried out on these topics before the development of hypertension. Nerve cell diameter, amount of Nissl granules in the cytoplasm and nucleus-to-cytoplasm area ratio, all well-known indicators of nerve cell activity in sympathetic ganglia, have been reported to be higher in SHR than those in normotensive Wistar-Kyoto (WKY) rats. This suggests hyperfunction of the peripheral sympathetic nervous system in SHR (15). These parameters, however, indicate the activity of individual ganglion cells, but not that of whole ganglia.

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Measurement of ganglion weight per body weight, and morphometrically examined ganglion cell volume and the relative area of ganglion cells in SCG and stellate ganglion (SG) of SHR, younger than 30 days of age and comparison to those of age-matched WKY rats were required (16). These authors showed that: (;') the ganglion weight/body weight ratios of SHR are significantly larger compared to those of WKY rats, within groups aged 30 days, and (/;') the ganglion cell volume and the relative area of ganglionic cells in both ganglia of SHR are significantly larger than those of age- matched WKY rats. These data suggest that the functional state of the whole SCG and SG is significantly higher in SHR than that in WKY rats already before the development of hypertension.

• **Protein and neurotransmitter synthetic activity of the ganglia**

Protein and catecholamine synthetic activities of ganglion cells of SCG and SG of SHR during the prehypertensive stage have been examined by light microscopic autoradiography with ^{3}H-lysine and ^{3}H-DOPA, respectively (17, 18). Silver grains representing toe localization of the labeled ^{3}H-lysine and ^{3}H-DOPA over the cytoplasm of ganglion cells in the SCG and SG have been counted in the same photographic magnifications. Significantly more silver grains over ganglion cells in SHR were observed as compared to age-matched WKY rats, using various incorporation times after injection of ^{3}H-lysine, in newborn and 30 days after birth groups. The increased incorporation of the label in both SCG and SG was more marked in newborn animals than 30 days old animals. These results confirmed that a hypersynthesis of protein in SCG and SG is present in SHR immediately after birth. Intravenously administered ^{3}H-DOPA is incorporated by nerve cells and rapidly metabolized intracellularly to catecholamines, such as dopamine, noradrenaline and adrenaline (19). Significantly greater number of silver grains were seen over ganglion cells of SCG and SG in SHR compared to those of age-matched WKY rats, using various incorporation times after injection of 'H-DOPA in age groups of 0 and 10 days (18). In newborn SHR, the sympathetic gan-

glion cells synthesized a larger amount of catecholamines which were transported or released more rapidly than in the SG cells of WKY rats. Hypersynthesis of catecholamine in the sympathetic ganglia in SHR has been established in this experiment. A summary of these morphometric analyses on protein and neurotransmitter synthetic activity of SCG and SG is given in Table 1. The total functional activity of SCG and SG as measured in these experiments was significantly higher in SHR than in WKY rats in the prehypertensive stage.
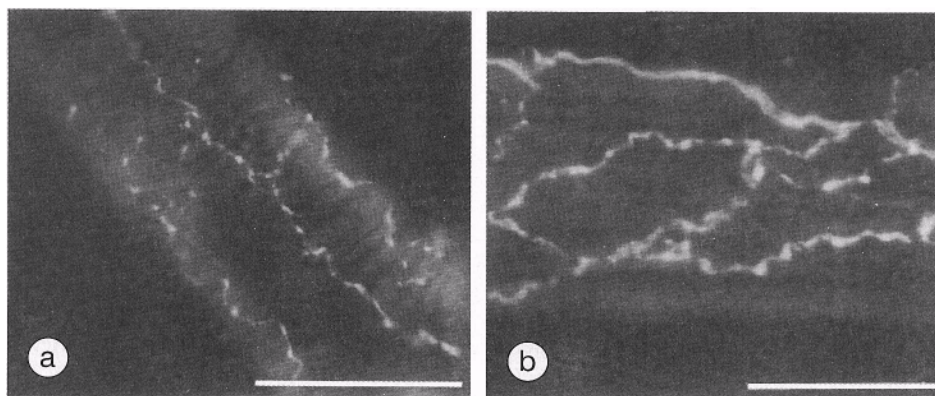
• **Noradrenergic innervation of the middle cerebral and coronary arteries**

The distribution of noradrenergic nerve fibers in the middle cerebral and coronary arteries of SHRSP has been examined by several authors to estimate the effect of SCG and SG hyperfunction. The distribution density of perivascular sympathetic nerve fibers in SHR has been investigated in various peripheral arteries such as the mesenteric (20), jejunal (21) and caudal arteries (22) in the prehypertensive (23-25), developmental and established stages (26, 27) of hypertension, using fluorescence microscopy (26), transmission (28) and scanning (29) electron microscopy. The glyoxylic acid method for the staining of fluorescent noradrenergic nerve fibers in whole mount preparations was applied (30, 31). The distribution densities of noradrenergic nerve fibers in the distal portions of the middle cerebral artery (Fig. 1) and in the coronary artery from the prehypertensive to the established hypertensive stage of SHRSP were significantly higher than those of age-matched WKY rats. The difference in the density of nerve fibers between SHRSP and WKY rats was higher in the prehypertensive stage than that in the established hypertensive stage.

Table 1. A comparison behveen spontaneously hypertensive rats (SHR) and Wistar-Kyoto (IVKY) rats in functional activity of superior cervical ganglia (SCG) and stelatte ganglia (SG)

Days after birth	0		10		30	
	SCG	SG	SCG	SG	SCG	SG
Relative weight[1]	↑	→	↑	↑	↑	↑
Ganglion cell volume	↑	↑	↑	↑	→	→
Relative area[2]	↑	↑	↑	↑	→	→
Protein synthesis	↑	↑	?	?	↑	↑
Neutotransmitter synthesis	↑	↑	↑	↑	↑	→
Total activity	↑↑	↑↑	↑↑	↑↑	↑	↑

T- *increase, —» - no significant difference, 1 - not examined, 1 - ganglion weight/body weight, 2 - area of ganglion cells/ area of whole ganglion tissue.*

Figure 1. Photomicrographs showing noradrenergic nerve fibers of the distal portion of the middle cerebral arteries of 180-day-old WKY (a) and SHRSP (b). Bars, 30 juin.

Increased sympathetic innervation of the distal portion of middle cerebral artery and of the coronary artery of SHRSP was assumed to be caused by hyperfunction of SCO and /or SG (16-18), because the perivascular sympathetic nerves innervating the major cerebral arteries originate from SCG (32, 33) and those innervating the coronary arteries originate from SG. Hyperfunction of SCG and SG in SHRSP may promote the development, elongation and branching of nerves supplying these arteries.

• **Noradrenergic innervation of the heart**

Although there were many data on the NA content in the heart of SHR from fetus to adult, no definite conclusion could be obtained. While some biochemical studies reported that NA levels in the heart in SHR and SHRSP were lower (9, 34-37) or not higher (38, 39) than those of WKY rats , others reported that NA content in the heart in SHR was greater than that in WKY rats (7). Nakamura *et al* (40) showed data that NA content in the heart was not significantly different between SHR and WKY rats at 6 weeks of age, but was lower in SHR than in WKY rats at 12 weeks of age. Watanabe *et al* (8), however, reported that there was no difference in NA content in the fetal heart between SHR and WKY rats, but NA content was higher in the heart of 16-week-old SHR than that of age-matched WKY rats. According to Adams *et al* (41), sympathetic activity was higher in SHR than in WKY rats in the left ventricle through most of the period between 4 and 50 weeks of age.

The noradrenergic nerve fiber density in the heart of SHRSP and WKY rats has been studied, using the glyoxylic acid method for staining frozen sections of m}ʳocardium and whole mount preparations of the subepicardium (42). The density of noradrenergic nerve fibers of the subepicardium of the right and left ventricles was higher in SHRSP as compared to that of WKY rats during 10 to 180 days of age. The density of the nerve fibers in myocardium of the right ventricle was higher in SHRSP as compared to that of WKY rats during 30 to 90 days of age. Noradrenergic nerve fiber density of myocardium in the left ventricle and in the ventricular septum of SHRSP was the same as that of WKY rats. These morphometric results suggested that the total amount of NA in the whole heart in SHRSP was larger than that of age-matched WKY rats up to six months after birth.

The hyperinnervation of the heart by noradrenergic nerve fibers in SHRSP is probably the primary change the heart goes through prior to develop an hypertension situation, which may be caused by hyperfunction of SG.

EFFECTS OF SYMPATHETIC HYPERINNERVATION ON THE BLOOD VESSELS AND THE HEART OF STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

• **Blood vessels**

Sympathetic nerves are known to affect vascular smooth muscle cells (SMC), both through direct, rapid action (43), and long-term regulatory and trophic effects (44,45). In SHRSP, the enhanced perivascular nerves may directly produce intensified vasoconstrictionby the increased NA release from their terminals, as is indicated by electric or K^f-stimulation (46-49), resulting in an increase or the maintenance of peripheral resistance. Sympathetic hyperinnervation of the blood vessels in SHRSP during the prehypertensive stage may, through trophic effects, provoke an elevation in protein synthesis in vascular SMC (50) and an augmentation of the arterial wall mass resulted from medial SMC hypertrophy (51-53) and/or hyperpla-

sia (54-56), although enhanced SMC proliferation is also re-ported to be genetically determined (54, 57). The thickened arterial media in the prehypertensive (23, 58) and established hypertensive stage (59) may become hyperreactive to contractile stimuli (60). In addition, vascular hypeiinnervation may also control the membrane properties of vascular SMC (61), which can induce supersensitivity to catecholamines (49, 62, 63). Thus the sympathetic hyperinnervation of blood vessels in SHRSP may produce structural and functional alterations in SMC (64) which tend to occlude the vascular lumen in resistant vessels. Therefore they cause an increase in peripheral resistance, an elevation of the blood pressure in the prehypertensive and developing stages of hypertension, and contribute to the maintenance of hypertension in the established stage.

From studies of noradrenergic nerve density it is concluded that hyperinnervation may play an important role in the development of hypertension in SHR and SHRSP (30). This is supported by the fact that the rise in blood pressure was effectively suppressed by sympathectomy in newborn SHR (55, 65-67).

• **Heart**

Sympathetic nerve fibers are involved in the development of cardiac muscle hypertrophy through a-adrenoceptors (68-70). Several studies have demonstrated that the a-adrenergic nerves stimulate protein synthesis of cardiocytes (68) and increase their size (71-73). Therefore, the higher density of the noradre-nergic nerve fibers in SHRSP compared to that of age-matched WKY rats may cause heart hypertrophy in SHRSP due to a primary alteration of the heart at the prehypertensive stage (74, 75). At the established hypertensive stage in SHRSP, the cardiocytes become further hypertrophied by high blood pressure as a secondary factor, because such a mechanical stretch stimulates the cardiocyte protein synthesis (76, 77). On the other hand, the complete abolition of the sympathetic activity in 4-week-old SHR prevented the development of cardiac hypertrophy (77). These data suggested the leading role of noradrenergic innervation in heart hypertrophy. Cardiocytes hypertrophy occurring as a primary and secondary change of the heart in SHRSP may in its turn augment cardiac activity, resulting in elevation of blood pressure in the prehypertensive and developmental stages of hypertension and in maintenance of hypertension in established hypertensive stage (77).

STRUCTURAL CHANGES IN THE SMOOTH MUSCLE CELLS OF THE MIDDLE CEREBRAL AND CORONARY ARTERIES IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT
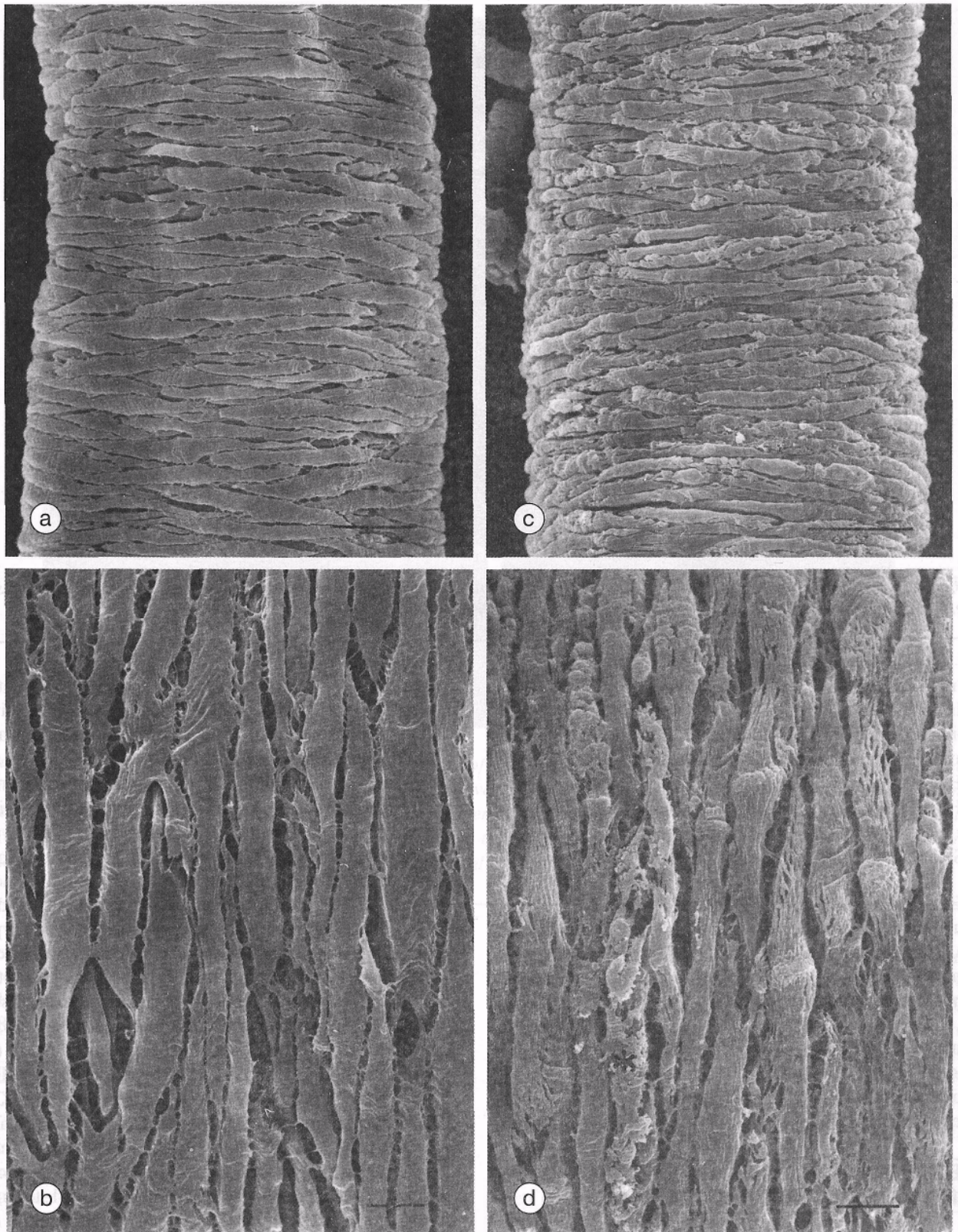
• **Middle cerebral artery**

The functional and structural alterations of blood vessels have been reported in SHR and SHRSP (78, 79). By scanning electron microscopy (SEM), the proximal portion of the middle cerebral artery of 6-month-old WKY rats has a compact and regular medial layer consisting of spindle-shaped SMC running almost parallel to each other and oriented transversely with respect to the long axis of the vessel. The SMC adventitial surface is smooth. Small pits with a round or elliptical openings are found only sporadically on the cell surface (Fig.2a,b). On the other hand, SMC in 6-month-old SHRSP exhibit a remarkably rough surface texture, though their arrangement is unaltered (Fig.2c,d). At a higher magnification, various structural modifications such as deep invaginations forming many pits and long longitudinal depression or grooves become visible on the surface of SMC in SHRSP (Fig.3). These structural modifications are often clustered, covering almost the whole cell width. Necrotic SMC constitute about 10% of all medial cells in SHRSP and no necrotic SMC were observed in WKY rats.

By transmission electron microscopy (TEM), SMC of the middle cerebral artery in WKY rats show the typical featur of SMC. In SHRSP, transverse profiles of SMC varied from rounded to irregular, with deep indentation of the plasma membrane. The SMC of SHRSP were surrounded by many layers of basal lamina-like material forming a labyrinth-like network in which electron-lucent amorphous material is found (Fig.4a,b). This finding may be consistent with previous biochemical studies that have demonstrated an increase in connective tissue fibers, collagen (80) and elastin (80, 81), in the arterial wall of SHR. Irregularly-shaped SMC contain all the organelles common to vascular SMC (82), indicating their ability to function normally.

• **Coronary artery**

By SEM, many pits and gutters are observed on the SMC surface of the coronary artery in both WKY rats and SHRSP at 4 months after birth. The SMC from 6-month-old WKY rats

Figure 2. Scanning electron microscopy images of the adventitial surface of medial smooth muscle cells in the proximal portion of the middle cerebral artery. Low power view of the outermost medial layer in WKY rat. The smooth muscle cells-are oriented transversely with respect to the vessel long axis (a). Smooth muscle cells from WKY rat are spindle-shaped with a smooth surface texture (b). Low power view of the outermost medial layer in SHRSP. Smooth muscle cells remain in a circular arrangement, but exhibit an irregular shape and rough surface texture (c). The smooth muscle cells from SHRSP exhibit a very rough surface texture. Asterisk shows a necrotic cell (d). Bar, 20 fim. x 740(a), Bar, 5 urn. x 2000 (b), Bar, 20 turn, x 900 (c), Bar, 5 fj,m. x 2200 (d).
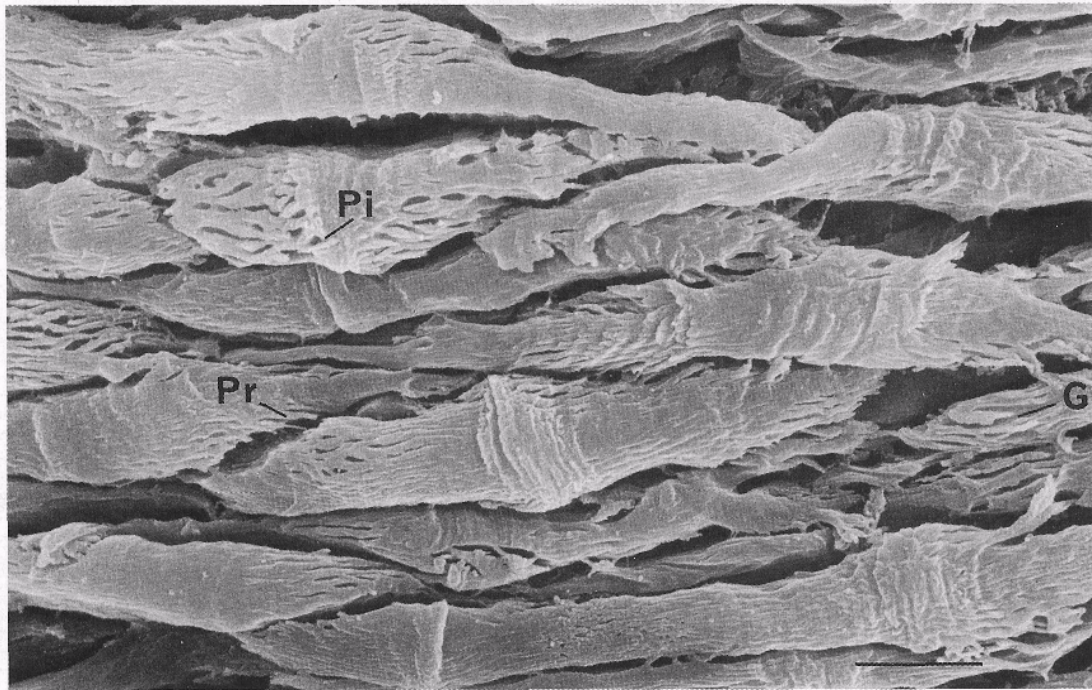
Figure 3. *Scanning electron microscopy image of smooth muscle cells of middle cerebral artery from SHRSP. The muscle cells form numerous pits (Pi), grooves (G) and processes (Pr). Bar, 5 pm.* x *3700.*

show rather similar surface texture to that of 4-month-old WKY rats. In contrast with WKY rats, SMC of 6-month-old SHRSP exhibit a remarkably increased number of gutters and pits on the surface. The intercellular space between SMC of 6-month-old SHRSP is increased in width compared to that of 4-month-old SHRSP and WKY rats and 6-month-old WKY rats. No necrotic cells, however, are found in the coronary artery of both SHRSP and WKY rats at these ages.

By TEM, the SMC of 6-month-old SHRSP and WKY rats are irregular in profile with deep indentations of the plasma membrane. They are surrounded by many layers of basal lamina-like material,. and large amount of collagen fibrils and fibrous matrix substances are observed between the SMC.

Taken together, the morphological alterations of vascular SMC of middle cerebral and coronary arteries in SHRSP may be adaptive changes to increased tension of the arterial wall in severe hypertension (83, 84).

PROTECTIVE ROLE OF SYMPATHETIC HYPERINNERVATION IN NECROSIS OF VASCULAR SMOOTH MUSCLE CELLS

•   Sympathetic hyperinnervation of blood vessels in SHR

and SHRSP has an important role in the development of hypertension. On the other hand, it may play a protective role against stroke due to a trophic effect on the vascular wall (85). Relation between the noradrenergic nerve fiber density and the structure of SMC in the middle, anterior cerebral arteries and in the ophthalmic artery, and the coronary artery, are investigate in both developmental and established stage of hypertension in SHRSP (86, 87). More than 10% of SMC in the proximal portion of the middle cerebral artery (86) and the distal portion of the anterior cerebral artery (unpublished data) of SHRSP become necrotic at the established stage of hypertension. These arteries were innervated by noradrenergic fibers of the same distribution density as those in WKY rats (30).' However, a few or no necrotic SMC are found in the distal portion of the middle cerebral artery (unpublished data), in the ophthalmic artery (87) and in the coronary artery (31) of SHRSP in the established stage of hypertension and all blood vessels were more heavily innervated than those in WKY rats. The difference in the occurrence of necrosis of vascular SMC may be attributed to the difference in nerve density. The latter suggests that sympathetic hyperinnervation may protect SMC from necrosis caused by the great tangential wall stress, which is associated with chronic hypertension. Such a kind of protective role of sympathetic nerves against SMC necrosis (and
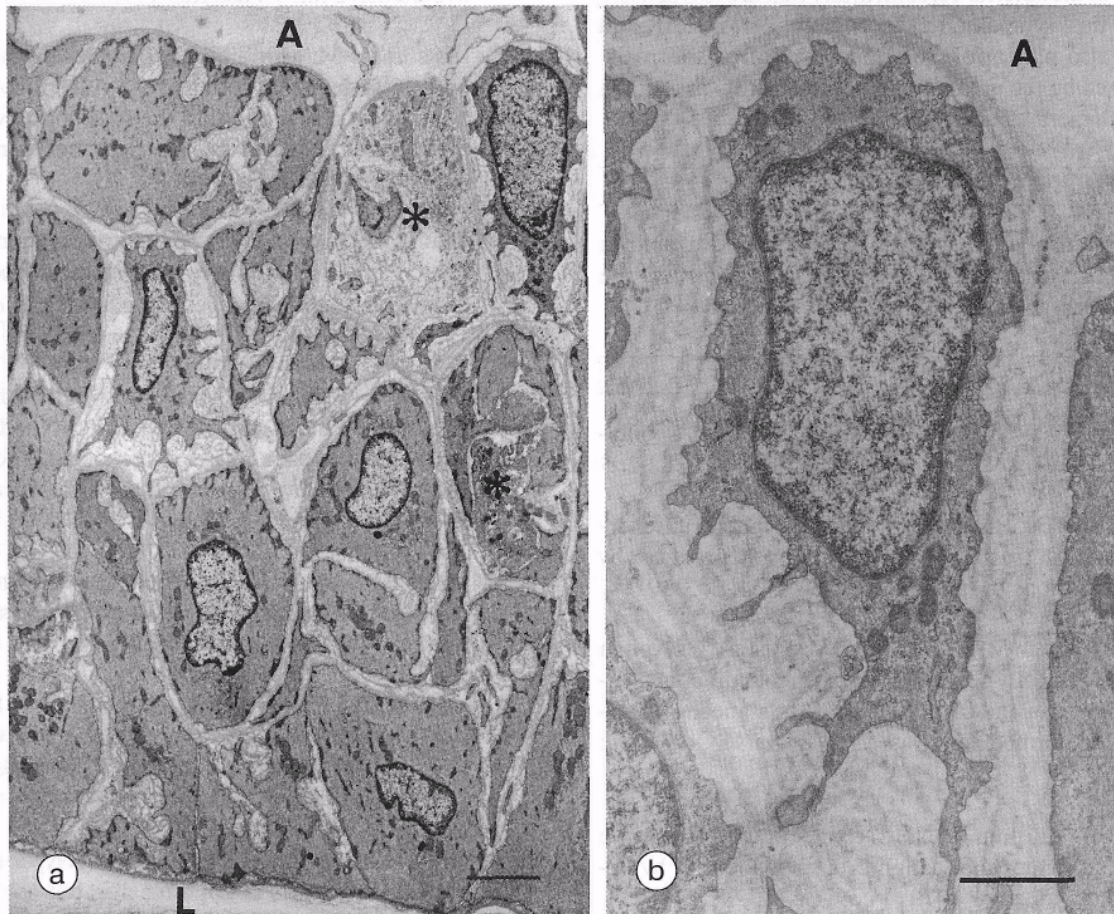
Figure 4. *Transmission electron micrographs of the media of proximal portion of SHRSP middle cerebral arteries sectioned parallel to the vessel long axis. The media consists of variously shaped smooth muscle cells, some are rounded and others are irregular with deep imaginations of the plasma membrane. Necrotic cells are depicted (asterisks). A - adventitial surface, L - lumlnal surface (a). Smooth muscle cell from the outermost medial layer exhibits an irregular profile and is surrounded by multiple layers of basal lamina-like material (b). Bar, 2 [im.* x 4900 (a). Bar, 1 /j.m.* x 16 000 (b).*

apoptosis ?) may be of a clinical value, since primary rupture of the cerebral arteries has been reported to be caused by "arteriosclerosis" accompanied by degeneration of the medial SMC(88,89).

CONCLUSION

• The relative ganglion weight, ganglion cell volume, relative ganglion cell area and protein and catecholamine synthetic activities of SCO and SG at prehypertensive stage of SHR are greater compared to those of age-matched WKY rats, suggesting a hyperfunction of SCO and SG in SHR before the hypertension development. The densities of perivascular norad-

renergic innervation in the distal portion of middle cerebral artery, the anterior cerebral, the ophthalmic and the coronary arteries and the heart, which are innervated by SCG or SG, are higher in SHRSP than those in WKY rats during the prehypertensive stage to the established hypertensive stage. Sympathetic hyperinnervation both in arteries and heart from SHR and SHRSP in the prehypertensive stage may have a leading role in development of hypertension, through SMC hypertrophy and hyperplasia and SMC hypersensitivity to NA.

Few necrotic SMC are seen in the densely innervated distal portion of the middle cerebral artery, the ophthalmic and the coronary arteries. On the other hand, many necrotic SMC are seen

in the proximal portion of the middle cerebral artery and the distal portion of the anterior cerebral artery that are less densely innervated. Thus, sympathetic hyperinnervation in SHR and SHRSP may also play a role to protect SMC from necrosis, which is caused by the great tangential wall stress associated with chronic hypertension.

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