Morphological Studies on the Autonomic Nervous System of Hypertensive Rats

I. Histometrical Study on the Superior Cervical Sympathetic Ganglion of Spontaneously Hypertensive Rats

MASAO MATSUMOTO

Department of Pathology, Faculty of Medicine, Kyoto University, Kyoto
(Director: Prof. Kozo Okamoto)

As generally accepted, hypertension is caused by arteriolar constriction which is responsible for the increase of peripheral vascular resistance. This arteriolar tone is regulated by the sympathetic nervous system. Maintenance of normal blood pressure requires a normal vasoconstrictor tone and this depends on tonic supplies of the sympathetic nervous system, arising from the brain stem, passing through the spinal cord and via peripheral nervous system to blood vessels, with modifications of moderator nerve reflexes and certain areas of the brain including the hypothalamus. A number of experiences has been reported concerning the production of high blood pressure in relation to the nervous system, clinically or experimentally, as reviewed by Tyler et al. Some concepts which manifested interests in neurogenic components of pathogenetical origin of hypertension, especially of "essential hypertension", have been presented, but there has been no definite interpretation up to the present time.

Pathophysiological studies on the autonomic nervous system in human essential hypertension have been carried out mainly in the form of functional study, and only a few investigations can be available on the morphological aspect. The difficulties in pathomorphological studies on human essential hypertension may be responsible for this fact; it may be practically impossible to get the materials which are not associated with any secondary modifications to this disease.

As already reported, spontaneously hypertensive rats (Okamoto and Aoki), which have been developed and maintained in our laboratory, may be considered to correspond to human essential hypertension owing to development of spontaneous hypertension without any treatment and the absence of remarkable pathological organ changes before the florid stage of hypertension. Extensive studies on the pathogenesis of hypertension in these animals have been performed mainly on the endocrine organs and the hypothalamus up to the present time.

Recently, in our laboratory, studies on the autonomic nervous system of the spontaneously hypertensive rats were set up from various aspects to clarify the relationship between nervous system and development and maintenance of hypertension in these animals. In this report the author investigated the superior cervical sympathetic ganglion histometrically, and tried to find out the pathogenetical roles.

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2. An outline of this study was reported at the 30th Annual Meeting of Japanese Circulation Society in March, 1966.
of the sympathetic nervous system in spontaneously hypertensive rats.

**Materials**

As materials, male spontaneously hypertensive rats (Okamoto and Aoki, abbreviated hereafter as S.H. rats) and male normotensive control rats, both separated from the Wistar strain, were used. The method of obtaining the S.H. rats was already reported\(^{21,22}\).

These animals were housed under constant temperature (22–24°C) and humidity (50–60 per cent), fed with stock chow diet* or Manitoba wheat mixed with dried sardine and seasoned with a little salt (both diets contained 0.3 per cent of sodium), and green vegetables. All rats were given tap water ad libitum.

The hypertensive animals were divided into three groups according to survival duration; that is, 1) those sacrificed in 40–60 days after birth, 2) in 4–6 months after birth and 3) in over 1 year (12–17 months) after birth. Each group corresponds to pre-hypertensive stage, initial stage of hypertension and advanced stage of hypertension, respectively. As for controls, normotensive rats of almost the same age and body weight as the hypertensives of each group were selected and used.

The numbers used for the animals in this study are as follows: (The details of source of the S.H. rats are as described in the preceding report\(^{29}\).) The S.H. rats of pre-hypertensive stage (T9123, T0241, T0242, 117001, 117002, 117007, 117009) and their controls (TT5, TT6, TT7, M–249, M–304, M–306, M–309); the S.H. rats of initial stage of hypertension (H9007, T9086, 9099, 9106, 10030, 10063) and their controls (5, 311, 312, 313, 323); the S.H. rats of advanced stage of hypertension (8100, 8102, 8107, 8109, T9001) and their controls (T54, T57, T58, T64, 60). The final blood pressure and body weight of these animals are as shown in Table I.

**Methods**

The blood pressure and body weight of all animals were measured at least once a week for 5 weeks of age to death. The modified tail-water plethysmographic method (Okamoto and Aoki\(^{21}\)) was employed for blood pressure determination.

Animals were sacrificed by decapitation early in the morning after 24 hours isolation. Immediately after sacrifice, the animals' superior cervical sympathetic ganglia were completely extirpated with great caution to avoid injury, removed from the surrounding tissues as perfectly as possible, and weighed with the single pan balance**, then fixed in 10 per cent formalin and embedded in paraffin by usual methods. From fixation to embedding these procedures were performed as rapidly as possible to prevent shrinking of the tissues. These embedded ganglia were sectioned serially in a thickness of 5 microns so as to make the cut-angle rectangular to the long axis of the ganglia, and the obtained sections were stained with hematoxylin-eosin method and Nissl stain with 0.1 per cent thionin. All these procedures were performed under identical conditions on all materials examined.

Histometrical examinations using thus stained serial sections, as well as expression of weight of the ganglion, were performed in the following manners. Besides, some histopathological changes were examined on the nerve cells in the same sections.

1. Weight of the ganglion

Weight of the ganglion was expressed as the ratio of actual weight to body weight;

\[ R_{wt} = \frac{\text{total weight of bilateral ganglia (mg)}}{\text{body weight (g)}} \times 100. \]

2. Volume of the ganglion

Dimensions of the cross-sections of the ganglion settled at a definite distance were measured and approximate value of volume of the ganglion (V) was calculated by the following formula;

\[ V = h \sum a_3, \]

where \( h \) is the distance between the settled sections (in this study, which was taken to be smaller than 50 microns throughout all cases) and \( a_3 \) is the dimension of each cross-section. Expression of volume was made as the ratio to body weight as follows;

\[ R_{vol} = \frac{\text{volume of ganglion (mm}^3\text{)}}{\text{body weight (g)}} \times 1000. \]

Measurement of dimensions of the cross-sections was performed planimetrically with magnification of 75–80 with the aid of the microprojector**. Those of the surrounding tissues or capsule of the ganglion and the bundle of nerve fibers, which could be histologically discriminated, were excluded from measurement.

3. Number of the nerve cells in the ganglion

Certain methods have been employed by some authors to examine the number of nerve cells in the ganglion\(^{35,36}\). In this study, however, the following method was applied. Among the same serial sections, several (5–10) cross-sections located in various parts of the ganglion were selected. On these sections, the number of the

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\* Oriental NMF, Oriental Yeast Co., Tokyo

\** Sartorius Series 2600, Sartorius-Werke AG., Göttingen

\*** Leitz Typ. XIC Xenon, Kr.00014, Ernst Leitz GMBH., Wetzlar

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nerve cells which showed clear-cut nuclear and cellular margin and revealed distinct nucleoli were counted, and the total number of the nerve cells in the ganglion (N) was computed by the following formula;

\[ N = \frac{\sum_{k=1}^{m} N_k}{m} \times V, \]

where \( V \) is volume of the ganglion, \( m \) is number of the sections on which the number of the cells was counted and \( N_k \) is number of the nerve cells in unit volume of the ganglion, which was calculated from each section as

\[ N_k = \frac{p_k}{a_k \times d}, \]

where \( p_k \) is number of the cells in each section, \( a_k \) is dimension of the section on which the number of the cells was counted and \( d \) is mean diameter of the nerve cells. This \( d \) was calculated approximately as

\[ d = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{x_i + y_i}{2} \right), \]

where \( x_i \) and \( y_i \) are the longest and shortest diameters of each cell and \( n \) is number of the cells examined.

This method is considerably simple but thought to be of practical use because distribution and size of the nerve cells are relatively uniform in the rat cervical sympathetic ganglion.

(4) Nuclear and cellular size of the nerve cells

Dimensional size of the cross-sectioned nuclei and cell bodies were measured in the same manner as mentioned in (2) with magnification of 1500–2000. In this study one hundred nerve cells were examined in each case. These were selected at random out of those which showed 1) clear-cut nuclear and cellular margin and 2) distinct nucleoli. The sections used were chosen so as to be separated from each other in more than 10 microns distance, for diameter of ganglionic nerve cells and their nuclei are about 15–25 microns and 10–15 microns, respectively. From the data thus obtained percentage distribution of nuclear and cellular size (size spectrum) was calculated and figured in individual cases, and compared with each other.

(5) Statistical Analysis

The data obtained by the above-mentioned methods in the S.H. rats and in the controls of each group were statistically analyzed using "\( t \)" test. The data were considered to be significant when the "\( p \)" value were less than 0.05.

RESULTS

The results obtained are shown in Table I, Fig. 1, 2, and 3.

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1. Blood Pressure and Body Weight

In the S.H. rats of 40–60 days of age average systolic blood pressure level (final) was 143±8 mm.Hg and those of individual animals were below 150 mm.Hg with the exception of 117002 and 117009. Since systolic blood pressure above 150 mm.Hg in rats may be considered as hypertension in this stage of hypertension was in the "pre-hypertensive" stage. But mean blood pressure level was slightly higher as compared with that of the controls even in this stage with a significant difference. Blood pressure of the S.H. rats in the initial and advanced stage of hypertension was always much higher than that of the controls.

Mean body weight of the S.H. rats seemed to be a little less than that of the controls in every stage in spite of the previously mentioned method of selection of animals. But no significant difference among them were proved statistically.

2. Weight and Volume of the Ganglion

The superior cervical sympathetic ganglia showed an increase in weight and volume as the rats grew older, both in the S.H. rats and in the normotensive controls.

Mean value of weight of the ganglia in the S.H. rats of pre-hypertensive stage (2.40±0.32) seemed to be larger than that of the controls (2.11±0.30), but no significant difference was proved. However, volume (3.86±0.35) was larger even in this stage than that of the controls (2.88±0.46).

In initial and advanced stage of hypertension the S.H. rats revealed significantly larger ganglia both in weight and volume (weight, 1.79±0.15 in initial stage and 1.60±0.17 in advanced stage; volume, 2.10±0.12 and 2.67±0.31) than the controls (weight, 1.23±0.09 and 1.22±0.16; volume, 1.71±0.15 and 1.99±0.42).

3. Number of the Nerve Cells in the Ganglion

There was a considerable disperse among the calculated cell numbers. This might be related to the problems of method for computation which was simplified as previously mentioned. But, looking at the present data, there was no difference between total cell numbers of the S.H. rats and those of the controls. Also, the nerve cells in the ganglia of these animals
## Table I Data of Histomterological Examinations on the Superior Cervical Sympathetic Ganglia of Control and Spontaneously Hypertensive Rats

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<th>Age</th>
<th>Kind of Animals</th>
<th>Animal No.</th>
<th>Final Blood Pressure (mmHg)</th>
<th>Body Weight (g)</th>
<th>Weight of Ganglion (mg)</th>
<th>Volume of Ganglion (mm³)</th>
<th>Number of Nerve Cells (μm²)</th>
<th>Mean Nuclear Size of Nerve Cells (μm²)</th>
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Note: * Spontaneously Hypertensive Rats

** \( R_{we} = \frac{\text{Weight of Bilateral Ganglia (mg)}}{\text{Body Weight (g)}} \times 100 \)**

*** \( R_{voe} = \frac{\text{Volume of Ganglion (mm³)}}{\text{Body Weight (g)}} \times 100 \)**

! significant (0.05 > \( P \) > 0.01)

!! \( u \) (0.01 > \( P \))

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Fig. 1. Nuclear and cellular size spectra of nerve cells in superior cervical sympathetic ganglia of spontaneously hypertensive rats of 40-60 days of age.
---; spontaneously hypertensive rats: ----; controls.

Fig. 2. Nuclear and cellular size spectra of nerve cells in superior cervical sympathetic ganglia of spontaneously hypertensive rats of 4-6 months of age.
---; spontaneously hypertensive rats: ----; controls.

Fig. 3. Nuclear and cellular size spectra of nerve cells in superior cervical sympathetic ganglia of spontaneously hypertensive rats of over 1 year of age.
---; spontaneously hypertensive rats: ----; controls.

Fig. 4. Photomicrograph of the nerve cells in the superior cervical sympathetic ganglion of the control rat and spontaneously hypertensive rat in pre-hypertensive stage.

*Right*: Control rat (M-249), 59 days after birth. Final blood pressure 104 mm.Hg, mean nuclear size 101.8 \( \mu^2 \), mean cellular size 296.2 \( \mu^2 \).

*Left*: Spontaneously hypertensive rat (117007), 60 days after birth. Final blood pressure 142 mm.Hg, mean nuclear size 128.5 \( \mu^2 \), mean cellular size 400.7 \( \mu^2 \). \( \times 400 \). Nissl stain.

Fig. 5. Photomicrograph of the nerve cells in the superior cervical sympathetic ganglion of the control rat and spontaneously hypertensive rat in advanced stage of hypertension.

*Right*: Control rat (60), 12 month after birth. Final blood pressure 116 mm.Hg, mean nuclear size 115.0 \( \mu^2 \), mean cellular size 409.2 \( \mu^2 \).

*Left*: Spontaneously hypertensive rat (T9001), 12 months after birth. Final blood pressure 170 mm.Hg, mean nuclear size 126.9 \( \mu^2 \), mean cellular size 487.0 \( \mu^2 \). \( \times 400 \). Nissl stain.

did not show any remarkable changes in number according to age.

4. Nuclear and Cellular Size of the Nerve Cells

Both nuclear and cellular size of the nerve cells in all animals increased up to the age of 4–6 months after birth, and, in the rats of over 1 year of age, the nuclei again diminished their size to almost the same extent as those of 40–60 days of age. Cell bodies, however, scarcely showed this diminution in size.

The percentage distribution of nuclear and cellular size of the nerve cells are illustrated in Fig. 1, 2 and 3. Fig. 4 and 5 show the photomicrographs of these nerve cells, where magnification is identical throughout all pictures.

Both nuclear and cellular sizes were obviously larger in the S.H. rats (nuclear size; 124.3 ± 7.1 μ² in pre-hypertensive stage, 139.9 ± 12.8 μ² in initial stage of hypertension and 129.5 ± 9.1 μ² in advanced stage of hypertension; cellular size; 387.9 ± 12.3 μ², 514.7 ± 26.1 μ² and 496.2 ± 54.1 μ², respectively) than in the controls (nuclear size; 103.4 ± 5.3 μ², 117.4 ± 11.9 μ² and 100.4 ± 9.3 μ²; cellular size; 319.1 ± 29.8 μ², 418.4 ± 22.0 μ² and 412.4 ± 11.2 μ²) throughout three stages with a significant difference. On the nuclear size, this difference was most remarkable in advanced stage, when it was 29 μ² and corresponded to about 29 per cent of the nuclear size of the controls, and in other stages, this was about 20 per cent. Cellular enlargement, on the other hand, was in the range of 20–22 per cent in all stages.

5. Histopathological Changes

Histologically the following findings were observed. First, Nissl granules seemed to be moderately abundant in the nerve cells of the S.H. rats as compared with those of the controls. These chromophilia were more remarkable in pre- and initial stages than in advanced stage of hypertension (Fig. 4 and 5). Second, such changes as peripheral chromatolysis or those similar to central chromatolysis, cellular ballooning or vacuolation, or nuclear shrinkage or eccentricity were found to some extent both in the S.H. rats and the controls. These changes, however, seemed to show no meaningful difference in degree among these animals.

**DISCUSSION**

As to the relationship between cellular activity and morphological changes of the cells a number of investigations have been carried out. As a matter of fact changes detected by histometrical examinations are not enough to understand cellular activity; together with this, some investigations such as enzyme-histochemical or electron microscopic study should be performed. On the nerve cells, however, some phenomena have been observed such as nuclear size changes by denervation in the neurons which were not affected and only in functional linkage, changes in nuclear size of the postganglionic cells induced by preganglionic stimulation or those of anterior horn cells of spinal cord under anesthesia. These might support the view that histometrical changes in the nerve cells could reflect neuronal activity to some extent and some authors applied this idea to their experiments. For example, WÜSTENFELD et al. proved that acoustic stimulations induce localized neuronal changes of the cochlea according to pitch, and SZENTAGOThAI et al. employed changes in nuclear size in the investigation of influences of the endocrine interferences on hypothalamic neuron activity. TALANTI et al. examined nuclear size of certain hypothalamic neurons in experimental hypertension, and IFT reported the nuclear size changes in the hypothalamic neurons induced by endocrine organ extirpations. All these were concerned with changes of the nuclei but those of the cell bodies may be thought to have identical meaning, not being so emphasized on the nerve cells. However, as mentioned by SZENTAGOThAI et al., considering only histometrical data, neither the direction (enlargement or shrinkage) nor the degree of the changes in size give a clew for the type of activity in which the nerve cells are involved.

In the present study the nuclear and cellular size changes were examined on the sympathetic ganglion cells, and enlargement of the nuclei or cell bodies was considered to show increased activities of the cells when the degenerative
or regressive processes were observed with the same or less frequency as compared with the normal subjects, though certain degenerative processes caused by cellular exhaustion might suggest existence of some stimulated or hyperactive states in former times\textsuperscript{37}. The author thinks, however, that a more extensive investigation which will cover the gaps between morphology and function of the cells should be performed to prove the validity of this concept.

Morphological studies on the autonomic nervous system of the subjects with hypertension, or with special reference to essential hypertension, have been reported by a few authors\textsuperscript{[14][15][38][36]}. These were, however, mainly concerned with histopathological changes of the sympathetic nerve cells and any available examinations applying the histometrical methods, which were performed in human essential hypertension as well as experimental hypertension, can not yet be found in the literature.

In 1947 Stöhr\textsuperscript{38} reported histopathological observation on the sympathetic ganglia of 5 patients with hypertension, but the renal hypertensives were involved in his materials. Nedzel (1951)\textsuperscript{38} also observed the sympathetic trunks of hypertensive man and dogs histologically. Histopathological study on the sympathetic ganglion cells in human essential hypertension was reported in 1955 by Walter et al.\textsuperscript{38}, who employed as the materials the lower thoracic and upper lumbar sympathetic ganglia extirpated together with splanchectomy for the purpose of surgical treatment of essential hypertension.

These authors recognized such pathological changes in the sympathetic ganglion cells as chromatinic abnormalities, deformation or eccentricity of the nuclei, ballooning of the cell bodies with cytoplasmic vacuolation or central chromatolysis (so-called “erschöpfte Zellen” (exhausted cells)\textsuperscript{38}), as well as hypertrophy and hyperplasia of the cellular processes and lumping of neurofibrils (“Fortsatzz-dysharmonie”\textsuperscript{38}), and these were observed more in the hypertensives than in the healthy subjects. From these findings they considered the probability of primary disorders in the sympathetic neurons as an etiological factor of essential hypertension.

Recently, Fujinawa\textsuperscript{14} reported the studies on the histopathological changes of the lateral horn cells of the spinal cord in 4 autopsied cases of essential hypertension. According to his observation the lateral horn cells showed such changes as chromatolysis, cell body swelling or nuclear eccentricity; all of these are axonal reactions and were revealed particularly remarkable in spinal renal vasomotor center or equally in whole lateral columns. He concluded, therefore, that some factors which induced the lateral horn cell changes such as granular proliferation in the radicular nerves were responsible for excessive vasoconstrictor flows, and that these changes might suggest a pathogenetical meaning of autonomic mechanisms in essential hypertension or participate in maintenance of hypertensive state.

In the present study the author examined the nerve cells in the superior cervical sympathetic ganglion of the spontaneously hypertensive rats (Okamoto and Aoki) histometrically. These S.H. rats have, as already mentioned, the characters which correspond to human essential hypertension and may be the most favorable materials for the experimental researchers to clarify the pathogenetical factors of essential hypertension. It is a matter of fact that only the findings observed in the superior cervical ganglia can not reflect the attitude of the sympathetic nervous system as a whole, but this may be one of the reliable sources on considering the sympathetic neural activity.

The results obtained by the present study revealed that 1) weight and volume of the ganglion were heavier and larger in the S.H. rats from initial stage and pre-hypertensive stage, respectively, and on the individual nerve cells in the ganglion, 2) both the nuclei and cell bodies were larger in the S.H. rats than in the controls from pre-hypertensive stage to advanced stage of hypertension with no significant changes of the nerve cell numbers in the ganglion, either among the animals of each experimental group, or according to age of the rats. The predominance in weight and volume of the ganglion of the S.H. rats may

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be explained by this superiority in size of the nerve cells involved in them rather than the numerical differences. In addition, histopathologically, scarcely any remarkable pathological, above all regressive or degenerative, changes of the nerve cells were observed in pre-hypertensive and initial stages of hypertension. Mild changes similar to such as chromatolysis with cellular enlargement, or nuclear shrinkage or eccentricity were found both in the hypertensives and normotensives of over 1 year of age. But severity or number of the affected cells were at most in the same range or even milder in the S.H. rats as compared with the normotensive controls. Therefore, in the present cases, it may be considered that these are incidental physiological phenomena to the course of aging of individual bodies, making allowance for the relations of these changes to cellular exhaustion. Besides, the amount of Nissl granules seemed to be more abundant in the S.H. rats, particularly in those of pre-hypertensive and initial stages, than in the controls. To this finding EINARSON’s interpretation that moderate chromophilia may point to initial activity of the nerve cells may be referred.

From the above-mentioned findings it is highly probable that the S.H. rats are in hyperactive state of the sympathetic nervous system, even in pre-hypertensive stage when the blood pressure is not so high as compared with the control normotensives, and this sympathetic hyperactivity may have some effective role on the development and maintenance of high blood pressure in these animals. The site where the source of this hyperactivity might be situated can not be analyzed only by the present work, but it may be mentioned that this is caused by rather qualitative, or functional, than quantitative changes of the constituents of the sympathetic nervous system. This seems to be reasonable considering that these two kinds of animals used in this study were separated from the identical strain.

On the pathogenesis of essential hypertension many investigations have been performed from various aspects and certain etiological factors, i.e. renal, endocrine or metabolic, neurogenic, or vascular, have been recognized. It is difficult, however, to establish the steadfast concept of etiology in this disease because of apparently equal strength of the rival theories. Some investigators believe the multiple interrelated factors as expressed in mosaic theory of PAGE. As previously stated extensive studies have been performed in our laboratory to throw light upon the pathogenetical factors of essential hypertension using excellent materials, the spontaneously hypertensive rats. The fact that the endocrine organs play a significant role in these animals was already reported. Moreover, recently, histochemical and histometrical studies were carried out on the various endocrine organs including adrenal medulla, and the hypothalamus of the S.H. rats. Also, the studies on the cardiovascular system of the S.H. rats were made pharmacodynamically. The results obtained by these investigations suggest that, in the S.H. rats, there exists excessive activity of the hypothalamic sympathetic zone-sympathetic nervous system, as well as the identity in the parasympathetics and hyperfunction of hypothalamus-pituitary adrenocortical or thyroid system, and these may result in the development of hypertension. The probable hyperactivity of the sympathetic nervous system in the S.H. rats, which was considered morphologically from the present study, seems to support well the above-mentioned findings.

Incidentally, the blood pressure levels of some hypertensives in advanced stage of hypertension were nearly as high as those in initial stage in spite of diminution in nuclear size or amount of Nissl granules together with mild degenerative changes of some nerve cells. Some organic changes in certain organs secondary to hypertension may be responsible for this finding.

In addition, to ascertain the above-mentioned interpretations, the same kind of examination on another parts of the sympathetic nervous system, or further investigations such as histochemical, ultrastructural, or biochemical and physiological studies on the autonomic nervous system, have to be made.

**Summary**

Histometrical studies were performed on
the superior cervical sympathetic ganglia of the spontaneously hypertensive rats (Okamoto and Aoki) and the following results were obtained.

1. Weights of the ganglia of the spontaneously hypertensive rats were significantly heavier than those of the control normotensives in initial and advanced stages of hypertension. The difference in mean value corresponded to 45 per cent of weights of the controls in initial stage and 31 per cent in advanced stage.

2. Volumes of the ganglia, and nuclear and cellular size of the nerve cells involved in them were significantly larger in the spontaneously hypertensive rats than in the controls from pre-hypertensive stage to advanced stage of hypertension. The volume of the ganglion of the hypertensives exceeded that of the controls by 34, 23 and 34 per cent in pre-hypertensive, initial and advanced stages, respectively. Those in nuclear size were 20, 19 and 29 per cent in respective stages, and cellular enlargement was in the range of 20–22 per cent throughout all stages.

3. Total numbers of the nerve cells in the ganglia revealed no significant difference among all materials examined.

4. Additional histopathological findings of the nerve cells showed moderate chromophilia and no meaningful degenerative changes in the spontaneously hypertensive rats as compared with the controls. The former was more remarkable in pre-hypertensive stage and initial stage of hypertension.

5. The probability of sympathetic hyperactivity in the spontaneously hypertensive rats was presumed from the above-mentioned results and discussed in relation to the recent studies in our laboratory.

REFERENCES


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