Morphological Studies on the Autonomic Nervous System of Hypertensive Rats*

III. Quantitative Estimation of the Cytoplasmic Structure of Neurons in Superior Cervical Sympathetic Ganglia of Spontaneously Hypertensive Rats

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AS ALREADY reported, some histometrical or enzyme histochemical observations on the superior cervical sympathetic ganglia of spontaneously hypertensive rats (OKAMOTO and AOKI)^{33,34} revealed the findings which might suggest the increased sympathetic neuronal activities in these animals^{21,22}. However, only one or two kinds of morphological observations may not be enough to lead to a conclusive result on such a functional problem as individual neuronal activity. From this point of view, the author has tried to make another kind of approach to find out a more decisive interrelation between morphology and functions, or to ascertain the interpretation on the probable sympathetic overactivity in the spontaneously hypertensive rats.

In the present study the author investigated the ultrastructure of neurons in the sympathetic ganglia of spontaneously hypertensive rats. As noticed also in the previous experiments, morphologically detectable changes in the sympathetic neurons were relatively small in the case of these animals, and, when grossly observed, it was somewhat difficult to find out any difference as compared with normal controls except in a few instances. Therefore, it was considered that a certain analytical procedure should be utilized which could be expected to disclose, if any, possible differences among the morphological findings, and the histometrical

method was applied in this study for analyzing the electron microscopic morphology of sympathetic neurons.

MATERIALS

Electron microscopic observations on the sympathetic neurons were carried out on a total of 18 male normotensive rats of Wistar strain and 18 male spontaneously hypertensive rats (abbreviated hereafter as S. H. rats). The S. H. rats were obtained by the usual method reported precedingly from our laboratory^{33,34} and, in the same manner used in the previous experiments^{21,22}, divided into 3 groups according to survival duration; i.e. (1) those sacrificed at 40 to 50 days after birth, (2) at 4 to 5 months after birth, and (3) over 1 year after birth. Each group corresponded to prehypertensive stage, initial stage of hypertension and advanced stage of hypertension in our designation, respectively³⁴. As for control rats, normotensive rats (the rats with blood pressure below 150 mm. Hg were considered as the normotensives) of almost the same age and body weight as individual S. H. rats, were selected and used. Some of these normotensive rats were those supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University, but most of them were their offspring bred in our laboratory.

The case numbers of the animals used in this study are as follows: The S. H. rats of 40 to 50 days of age (147034 ♂, 147035 ♂, 147039 ♂, 157001 ♂, 157002 ♂, 157003 ♂) and their control rats (724 ♂, 725 ♂, 728 ♂, 729 ♂, 730 ♂, 731 ♂); the S. H. rats of 4 to 5 months of age (147024 ♂, 147025 ♂, 147026 ♂, 147027 ♂, 147031 ♂, 147032 ♂) and their controls (701 ♂, 702 ♂, 703 ♂, 704 ♂, 707 ♂, 711 ♂);

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the S. H. rats of over 1 year of age (147005 ♂, 147009 ♂, 147011 ♂, 147016 ♂, 147020 ♂, 147023 ♂) and their controls (645 ♂, 646 ♂, 647 ♂, 648 ♂, 649 ♂, 650 ♂). Among these S. H. rats F₁₅ rats (157001 ♂, 157002 ♂, 157003 ♂) were bred from F₁₄ animals (140150 ♂, 140157 ♀), and F₁₄ rats (147005 ♂, 147009 ♂, 147011 ♂, 147016 ♂, 147020 ♂, 147023 ♂, 147025 ♂, 147026 ♂, 147027 ♂, 147031 ♂, 147034 ♂, 147035 ♂, 147039 ♂, 140150 ♂, 140157 ♀) were obtained by matings of F₁₃ rats (130035 ♂, 130036 ♂, 130037 ♂, 130038 ♀, 130039 ♀). These F₁₃ rats were bred from F₁₂ animals (120041 ♂, 120054 ♀), and the details of source of these F₁₂ animals were already reported in the preceding papers^{33,34}.

All these animals were housed in the animal room with completely controlled air-conditioning (room temperature and humidity were set in the range of 22 to 24°C and 50 to 60 per cent, respectively) and artificial lighting. They were fed on stock chow diet* and green vegetables. Tap water was given for drink ad libitum.

METHODS

Blood pressure and body weight of all animals were measured as a rule once a week from 5 weeks of age all through life, and checked 3 or 4 times continuously prior to sacrifice. For blood pressure determination the modified tail-water plethysmographic method (OKAMOTO and AOKI³³) was employed.

Preparation of the materials for electron microscopy Animals were sacrificed early in the morning after 24 hours' isolation in individual cages. Immediately after sacrifice of the animal the superior cervical sympathetic ganglion was removed as rapidly as possible (extirpation of ganglion was performed within 1 minute following sacrifice), cut into 3 or 4 pieces and fixed at 0 to 4°C with 6.5 per cent glutalaldehyde in 0.1 M phosphate buffer adjusted to pH 7.4 (after SABATINI et al.⁴⁶). The tissue was removed from the fixative after 2 hours, washed and soaked in 0.1 M phosphate buffer (pH 7.4) at 4°C for 12 hours, and then transferred to 1 per cent OsO₄ in Veronal-acetate buffer at pH 7.4 (after PALADE³⁸). In this OsO₄ solution 0.045 g of sucrose per one milliliter of solution was added to increase tonicity (after CAULFIELD⁴). After 2 hours' fixation at 4°C, the tissue was washed in the same buffer as that used in OsO₄ fixation, dehydrated through a series of graded ethyl alcohol, soaked in transitional solvent, propylene oxide, and embedded in epoxy resins. The mixture of the following composition was used

for embedding: Epon 815, 7.0 ml; Epon 812, 3.0 ml; dodecynyl succinic anhydride, 14.7 ml; tris (dimethylaminomethyl) phenol (DMP 30), 0.5 ml. Ultrathin sections were made with a Porter-Blum ultramicrotome**, and stained with uranyl acetate (after WATSON⁴⁹) for 10 minutes following Millonig's lead solution²³ for 5 minutes.

All these procedures were performed under strictly identical conditions throughout whole materials.

Electron microscopic observation and analysis of cytoplasmic structure Ultrathin sections of sympathetic ganglion prepared as above-mentioned were examined and photographed in JEM-7A electron microscope***.

Quantitative cytoplasmic structure analysis in thin section was performed principally after the techniques described by LOUD¹⁹ and LOUD et al.²⁰. At first, a total of 6 or 7 sections were selected out of those cut from several tissue blocks of an animal, eliminating apparently inadequate ones for ultrastructural examination. After gross observation with electron microscope throughout these sections the neurons whose cytoplasmic structure was to be examined were selected at relatively low magnification (less than $\times 2500$); under such conditions structural details could not be clearly discriminated in electron microscopic images and a mistake through bias in selection could be avoided. At the same time caution was observed in selecting the cells in which nuclei and nucleoli were visible and nucleus-cytoplasmic ratios seemed to be almost identical. The neurons were initially microphotographed at a magnification of $\times 2000$ or $\times 2500$, dividing the whole cellular area into several parts, when necessary. These photographs were enlarged so as to make prints with final magnification in a range of $\times 10000$ to $\times 12500$, and the whole cellular configuration of nerve cell was reconstructed on a paper by assorting the micrographs on which cellular images were separately taken.

Measurement of the cytoplasmic structure was made by the following manners on thus prepared electron microphotographs of sympathetic nerve cells. At first, fine parallel lines (sampling lines) spaced 10 mm apart were drawn over the photograph of a cell, and total length of sampling lines which actually lay on the picture of cell body was measured by the millimeter scale of a ruler. Then, two kinds of measurements were performed along these sampling lines; (1) cumulative measurement of length of the lines that passed over defined figures of various cytoplasmic structures (formed bodies), and (2) counts of the number of intersections be-

* Oriental NMF (Oriental Yeast Co., Tokyo, Japan), and CLEA CA-1 and CE-2 (Japan CLEA Co., Tokyo, Japan).

** Porter-Blum Ultra-microtome MT-2, Ivan Sorvall, Inc., U. S. A.

*** JEM-7A 10138-91, Japan Electron Optics Laboratory Co., Ltd., Japan.

tween the superimposed lines and the profiles or edges of some specific membranous structures. As to the former, among various cytoplasmic structures, mitochondria, lysosomes and free ribosomes were considered as formed bodies, and areas occupied by granular endoplasmic reticulum (Nissl body) and Golgi apparatus were also measured in like manner. Measurement of fractional lengths of the sampling lines was made individually on these structures, and calculation was performed on percentage ratios of the total length of sampling lines occupied by each of nucleus and cytoplasm to those by whole cell body, and percentage ratios of the sampling lines occupied by each of the cell organelles to those traversing cytoplasm. By the principle of DELESSE⁸, if the statistically large and unbiased samples were examined, it is possible to equate the fractional length of the total lines traversing a given component with the fractional volume of it in the sectional tissue. Therefore, the percentage value calculated above is thought to represent percentage volume of

cell body or cytoplasm, which was occupied by each cellular structure. In reference to (2), the following calculation was made. According to the description of LOUD et al.²⁰ length of profiles or edges of cytoplasmic membranous structures in microns per square microns of sectioned tissue surface can be obtained by the following;

$$\frac{\pi C}{2L} \left(\frac{M}{1000} \right),$$

where C is the number of crossings of the sampling lines and the membrane profiles on photographs, M is magnification and L is total length of the sampling lines traversing cytoplasmic area. LOUD et al. defined this as "membrane profile concentration". By multiplying this value by $4/\pi$, surface dimension of membranous structures in square microns per cubic microns of cytoplasmic volume can be calculated. In this study, the author applied this value to quantitative estimation of such membranous structures as granular endoplasmic reticulum or Golgi apparatus.

TABLE I MATERIALS USED FOR ELECTRON MICROSCOPIC HISTOMETRY ON THE NEURONS IN SUPERIOR CERVICAL SYMPATHETIC GANGLIA OF SHR* AND NORMOTENSIVE CONTROL RATS

Age	SHR*			Normotensive Control Rats		
	Case No.	Final Systolic Blood Pressure (mm. Hg)	Body Weight at Sacrifice (gm)	Case No.	Final Systolic Blood Pressure (mm. Hg)	Body Weight at Sacrifice (gm)
40-50 Days	147034	144	142	724	120	138
	147035	150	114	725	116	125
	147039	136	132	728	118	127
	157001	136	118	729	120	125
	157002	140	100	730	116	132
	157003	154	118	731	120	118
	<i>MV</i> ± <i>SD</i> **	143.3 ± 8.9"	120.7 ± 14.7	<i>MV</i> ± <i>SD</i> **	118.3 ± 2.0"	128.0 ± 6.9
4-5 Months	147024	206	264	701	116	365
	147025	180	250	702	120	345
	147026	180	320	703	108	316
	147027	190	282	704	104	260
	147031	184	280	707	112	248
	147032	192	326	711	118	330
	<i>MV</i> ± <i>SD</i> **	188.7 ± 10.0"	287.0 ± 29.2	<i>MV</i> ± <i>SD</i> **	113.0 ± 6.2"	310.7 ± 47.2
Over 1 Year	147005	190	405	645	100	406
	147009	206	452	646	112	432
	147011	180	468	647	122	412
	147016	190	394	648	110	418
	147020	194	392	649	112	398
	147023	200	384	650	128	350
	<i>MV</i> ± <i>SD</i> **	193.3 ± 7.6"	415.8 ± 32.0	<i>MV</i> ± <i>SD</i> **	114.0 ± 9.0"	402.7 ± 26.8

* Spontaneously Hypertensive Rats " Statistically Significant ($0.01 > P$) ** Mean Value ± Standard Deviation

The data for each nerve cell were recorded on data sheets as seen in Table II. Six cells were selected in each animal and consequently a total of 36 neurons per each experimental group were examined by

TABLE II AN EXAMPLE OF DATA SHEET USED FOR LINEAR ANALYSES OF THE CYTOPLASMIC STRUCTURE OF RAT SYMPATHETIC NEURONS

Plate No.: 3227. 3228 Print Magnification (M): 10,000

	L (mm)	Nucleus (mm)	Cytoplasm (mm)								IS
			Total	Formed Bodies			Membrane Space				
				Mit	Lys	fR	gER	c	Go	c	
1	149		149	4	4	10	22	12			
2	159		159	9		8	71	22			
3	173		173	6		1	70	20			
4	188		188	13		3	70	32			
5	205		205	12	4	18	65	18			
6	223		223	5	8	6	37	30	40	46	
7	228	30	198	15	13	9	36	16			
8	234	68	166	10		5	33	18			
9	241	89	152	5		9	41	22			
10	252	112	140	7	5		47	32			
11	260	119	141	14			38	22			
12	276	126	150	15		2	42	15			
13	318	129	189	7		7	26	16			
14	323	180	193	20		17	26	8			
15	323	123	200	22		3	33	22	6	8	
16	323	128	195	25		9	30	14	7	11	
17	325	131	194	6	4	2	16	12	18	24	
18	321	125	196	9		6	31	16	7	12	
19	331	119	212	9	4	7	38	16			
20	342	113	229	17	9	4	25	22			
21	336	102	234	19	7	13	39	20			
22	335	79	256	26	7	10	21	16			
23	327	34	293	22	10	2	81	44			
24	334		334	6		17	58	36	10	19	
25	335		335		9	4	85	40	15	17	
26	333		333	26	4	15	96	38	26	33	
27	329		329	27	8	7	120	48	10	17	
28	325		325	18	6	17	101	48	25	50	
29	318		318	8	12	18	98	36	31	37	
30	307		307	12	3	16	81	44	22	40	
31	296		296	7	2	16	90	42	11	17	
32	287		287	15	5	10	84	40	28	42	
33	272		272	6	32	3	128	60	12	25	
34	260		260	10	10	12	104	52	15	22	
35	257		257	18		9	40	16	30	20	
36	250		250	2		9	86	48	35	36	
37	238		238	26	7	2	108	46	12	19	
38	222		222	14	5	12	74	16	20	37	
39	216		216	12	8	7	93	42			
40	194		194	14	4	10	80	22			
41	169		169	3	6	7	17	12			
42	136		136	20		11	7	6			
43	85		85		4	6					
44	55		55	5		7					
45	44		44	1	3	1					
46	43		43								
47	25		25								
Total	11522	1757	9765	548	203	367	2488	1157	380	532	4090
% Cellular Volume		15.2	84.8								
% Cytoplasmic Volume				5.61	2.07	3.76	25.47		3.89		59.20
Ratio of Square μ Surface to Cubic μ Cytoplasm								2.30		1.08	

Abbreviations:

L: Length of the Sampling Line Traversing Cellular Area; Mit: Mitochondria; Lys: Lysosomes;
fR: Free Ribosomes; gER: Granular Endoplasmic Reticulum; Go: Golgi Apparatus;
IS: Interspace; c: Numbers of Crossings of the Sampling Line with Membrane Profiles.

the above-mentioned analytical procedure.

Statistical analysis of data was performed by student "t" test for 95 per cent confidence limit ($P < 0.05$).

RESULTS

Final blood pressure level and body weight at sacrifice of the animals were as illustrated in Table I. Almost the same tendency as those shown in the previous reports^{21,22} were observed also in this study.

General observations The ultrastructural feature of the constituents of sympathetic ganglion in normal adult rat did not differ basically from the existing descriptions on those of rat or other mammals. Under low magnification many nerve cells of various sizes were observed in the matrix which consisted of collagen fibrills and non-neuronal cells, and each of them was enveloped with continuous cell layer of the satellite cells. Unmyelinated nerve fibers and sometimes myelinated fibers were seen in cer-

tain areas. The sympathetic nerve cell body showed various submicroscopic structures which can be observed in the neurons of another nervous system. The ganglion cell was comparatively large and had numerous branched dendrites and an axon. The nucleus usually showed spherical configuration and contained one or two distinct round nucleoli. The nuclear envelope consisted of a pair of membranes with numerous pores, and the nucleoplasm contained electron dense granules in clusters. The perikaryon showed an assortment of various cell organelles. A most characteristic feature was the widely distributed granular endoplasmic reticulum, consisting of isolated and relatively short pairs of membranes surrounded and separated by abundant small dense granules (ribosomes), which occurred in rows along the cytoplasmic surface of membranes or sometimes aggregated in the form of a small rosette. Free ribosome particles were also found forming group not attached to endoplasmic reticulum

Fig. 1. Single sympathetic ganglion cell of a normotensive rat (No. 724, 49 days old, male).
N: nucleus, Nl: nucleolus, Sat: satellite cell, F: nerve fibers.

membranes. Mitochondria also occurred throughout the perikaryon. They appeared to be rod-like or small spherical, and sometimes branched, and possessed internal structures similar to those found in other cell types. Some of them seemed to swell up slightly or showed a cristolytic tendency. Distinct Golgi apparatuses were found mainly in the inner half of cytoplasm, showing in sections the scattered profiles of stacked vesicles and cisternae. In the neighbourhood of the nucleus certain areas containing some agranular endoplasmic reticulum, dense bodies and numerous small vesicles were seen; they were thought to be correspond to GERL complex termed by NOVIKOFF²⁸. Besides, electron dense materials occurred in a form of scattered round, oval or sometimes somewhat irregularly shaped bodies in other

areas of perikaryon. They consisted of homogeneous or granular masses, and sometimes had clear limiting membranes and signs of lamellar internal organization or vacuolation. These structures were interpreted as lysosomes, and those found in the perinuclear zone seemed to have close relation to endoplasmic reticulum in regard to their origin. Multivesicular bodies were also found in certain numbers. They were observed as a small group of vesicles surrounded by a single limiting membrane, and dense inclusions or membranous structures were contained in some of them. The cytoplasmic structure which occurred in far less frequency than the above-mentioned was concentrically laminated membranous profile and looked like an onion slice. This kind of structure was already described in the reports of PALAY and PALADE⁴⁰

Fig. 2. Cytoplasmic structure of sympathetic ganglion cell of a normotensive rat (No. 707, 5 months old, male).
 Ni: granular endoplasmic reticulum (Nissl body), Mit: mitochondrion,
 Go: Golgi apparatus, Ly: lysosome.

or FORSSMANN¹³, and a similar structural organization was found in this study. Centrioles were found also in sympathetic neurons but only in a few cells examined, and this might be related to the absence of mitotic activity in neurons. The other intervening areas or matrix of the perikaryon showed relatively low electron density, and some fine filamentous structures were seemed to traverse certain regions of the cytoplasmic matrix. In addition, slender processes of satellite cells were found in the neuronal cytoplasm, appearing to either penetrate the cytoplasm or invaginate deeply at the surface abutting the neuron. The nerve terminals were also found just outside the neuron body, and the synaptic structure of these regions showed characteristic features described already by many investigators; the details will be omitted here.

Although the cytoplasmic ultrastructure of sympathetic neurons described above was that observed in normal adult rats, no remarkable difference could be found in rats at 40 to 50 days of age by such a comparatively gross inspection. The rats of over 1 year of age also showed almost the same pictures as the former two groups, despite that only lysosomes were seemed to increase in them to a certain extent as compared with those in younger ones.

In the S.H. rats, the fundamental structural organization of ganglionic neurons did not differ from that of the normal rats, and any peculiar structures which might be considered as being characteristic in the S.H. rats could not be found. By general observation, however, granular endoplasmic reticulum or Nissl bodies were thought to be more abundant in the S.H. rats of all ages in a certain degree as compared with the normal rats, just like that noticed in the light microscopic observation²¹. Mitochondria also appeared to be dominant in some cases of the S.H. rats, not being apparently confirmable by the reason that they occupied relatively small parts in the total cytoplasmic area.

Quantitative estimation of the cytoplasmic ultrastructure The results of the cytoplasmic structure analyses are illustrated in Table III and Table IV. Table III shows per cent ratios of the nucleus and the cytoplasm to whole cell volume, and the calculated volumes of cyto-

plasmic structures indicated by per cent ratios to total cytoplasm. As described already, to make materials uniform, the nerve cells to be examined were selected out of those whose nucleus-cytoplasmic ratios seemed to be almost equal. This was confirmed by calculation of per cent ratios of nuclear and cytoplasmic volumes as seen in Table III.

In the cytoplasmic structures the granular endoplasmic reticulum covered a wider sphere in the S.H. rats than in the normotensive control rats through all ages. Free ribosome areas also tended to be larger in the S.H. rats. These results corresponded to the finding obtained by gross observation. Roughly estimated, the granular endoplasmic reticulum of neurons in the S.H. rats was about one and a half times larger than that in the controls. At the same time, the granular endoplasmic reticulum seemed to be more abundant in the younger S.H. rats than in the older ones.

Total mitochondrial volume was greater in the S.H. rats in comparison with that of the normotensives, and the lysosome particles also proved the same feature. However, there was a disparity between them; that is, the former showed no remarkable difference among the S.H. rats of all ages and the latter tended to increase especially in the older ones. Some of the mitochondria in the neurons of S.H. rats showed swelling or cristolysis to some extent, appearing to be a little marked as compared with those in normotensives.

As to the Golgi apparatus, statistically significant predominance was observed only in the S.H. rats of prehypertensive stage, though a similar tendency was also seen in those of other age groups.

Table IV illustrates the calculated surface dimensions of the membranous cytoplasmic structures (granular endoplasmic reticulum and Golgi apparatus). While approximate volumes occupied by these structures were computed as mentioned above, these estimations might be somewhat poor in exactness due to their structural complexity. Calculation was made, therefore, on the surface dimension of these structures, and the results showed, as seen in Table IV, predominance of the granular endoplasmic reticulum in the S.H. rats of all ages and that

Fig. 3. Submicroscopic feature of the cytoplasm of sympathetic ganglion cell in a spontaneously hypertensive rat (SHR, No. 157002, 48 days old, male) and a normotensive control rat (NR, No. 730, 50 days old, male).

TABLE III AVERAGE VALUES OBTAINED BY LINEAR ANALYTICAL EXAMINATIONS ON THE ELECTRON MICROGRAPHS OF NEURONS IN SUPERIOR CERVICAL SYMPATHETIC GANGLIA OF SHR* AND NORMOTENSIVE CONTROL RATS

Age	Kind of Animals	Animal No.	Nucleus and Cytoplasm (Per Cent of Total Cellular Volume)		Cytoplasmic Structures (Per cent of Total Cytoplasmic Volume)					
			Nucleus	Cytoplasm	Formed Bodies			Membrane Space		Interspace
					Mito- chondria	Lyso- somes	Free Ribo- somes	Granular Endoplasmic Reticulum	Golgi Apparatus	
40-50 Days	SHR*	147034	10.4	89.6	6.76	2.24	8.19	30.37	4.36	48.08
		147035	11.3	88.7	8.05	3.61	5.71	38.69	5.92	38.02
		147039	11.3	88.7	7.83	3.40	5.80	32.36	5.84	44.77
		157001	10.4	89.6	8.85	3.17	4.55	34.77	5.91	42.74
		157002	12.4	87.6	8.02	4.05	5.45	35.17	5.39	41.92
		157003	14.4	85.5	7.91	2.49	6.18	34.86	5.43	43.13
		MV± SE**	11.7± 0.62	88.3± 0.62	7.90± 0.27"	3.16± 0.28'	5.98± 0.47'	34.37± 1.15"	5.49± 0.24"	43.90± 1.40"
	Control	724	10.2	89.8	4.70	2.12	4.37	21.95	3.90	64.96
		725	10.3	89.7	4.65	2.40	4.06	22.06	4.43	62.40
		728	11.0	89.0	4.52	1.92	4.35	23.28	4.11	62.82
		729	10.3	89.7	4.96	2.28	4.21	22.92	4.57	61.06
		730	11.7	88.3	5.06	2.07	4.10	22.18	4.42	62.17
		731	12.8	87.2	5.12	2.18	4.68	22.43	4.62	60.97
		MV± SE**	11.1± 0.33	88.7± 0.33	4.50± 0.18"	2.16± 0.07'	4.30± 0.09'	22.47± 0.21"	4.34± 0.11"	62.38± 0.62"
4-5 Months	SHR*	147024	14.4	85.6	9.29	2.14	5.11	26.33	3.51	53.62
		147025	13.3	86.7	7.37	2.43	3.28	32.47	4.29	50.16
		147026	15.3	84.7	8.29	2.55	3.75	34.42	5.03	45.96
		147027	14.0	86.0	7.68	2.73	3.23	32.28	4.54	49.44
		147031	14.2	85.8	7.06	2.12	3.34	32.60	3.87	52.01
		147032	14.6	85.4	9.38	2.31	5.36	30.03	5.36	47.56
		MV± SE**	14.3± 0.27	85.7± 0.27	8.18± 0.40"	2.38± 0.10"	4.01± 0.38	31.21± 1.11"	4.43± 0.28	49.79± 1.15"
	Control	701	13.5	86.5	5.15	1.52	3.88	18.48	4.09	66.88
		702	10.8	89.2	4.32	1.80	5.63	19.02	3.65	65.58
		703	13.4	86.6	6.16	1.27	2.09	18.16	5.04	67.28
		704	16.9	83.1	5.76	1.53	3.31	24.66	3.83	60.91
		707	15.2	84.8	5.61	2.07	3.76	25.47	3.89	59.10
		711	10.3	89.7	5.61	1.53	2.26	16.42	2.10	72.11
		MV± SE**	13.3± 1.00	86.7± 1.00	5.44± 0.26"	1.62± 0.13"	3.48± 0.52	20.35± 1.64"	3.77± 0.39	65.34± 1.93"
Over 1 Year	SHR*	147005	10.5	89.5	6.99	4.39	5.67	31.39	5.53	46.03
		147009	10.8	89.2	8.34	3.87	5.13	30.73	5.30	46.63
		147011	9.4	90.6	8.66	4.70	6.07	30.45	5.92	44.20
		147016	10.3	89.7	7.41	4.34	5.34	26.45	4.66	51.80
		147020	13.4	86.6	8.34	4.91	5.69	30.48	6.57	44.01
		147023	7.9	92.1	7.17	4.07	4.46	29.41	6.07	48.82
		MV± SE**	10.4± 0.74	90.6± 0.74	7.82± 0.29"	4.36± 0.16"	5.39± 0.23"	29.82± 0.73"	5.69± 0.28	46.92± 1.21"
	Control	645	10.0	90.0	4.36	2.36	3.45	22.54	4.36	62.93
		646	10.6	89.4	4.43	2.35	3.48	23.17	4.75	62.82
		647	9.9	90.1	5.49	2.67	4.27	23.10	4.30	60.17
		648	9.4	90.6	5.19	2.60	4.14	19.62	5.97	62.48
		649	10.1	89.9	5.36	2.71	4.26	20.56	5.54	61.57
		650	10.4	89.4	4.64	2.56	4.74	20.22	4.09	63.75
		MV± SE**	10.1± 0.17	90.9± 0.17	4.91± 0.20"	2.54± 0.06"	4.04± 0.21"	21.54± 0.64"	4.81± 0.26	62.16± 0.64"

* Spontaneously Hypertensive Rats.

' Statistically Significant (0.05>P>0.01).

** Mean Value±Standard Deviation

" Statistically Significant (0.01>P).

→ Abundance of granular endoplasmic reticulum and mitochondrial predominance can be noticed in the neuron of a spontaneously hypertensive rat. N: nucleus, Ni: granular endoplasmic reticulum, Mit: mitochondion, Go: Golgi apparatus, Ly: lysosome.

of Golgi apparatus in the rats in prehypertensive stage. Also here the above findings were confirmed.

DISCUSSION

In the present study electron microscopic observations were performed on the ganglionic neurons of S.H. rats and normotensive control

rats, utilizing linear analytical procedures for morphological evaluation of the cytoplasmic ultrastructure. As seen in the methodological description and consequently in the results, the observation was somewhat gross or rough in character for electron microscopy. However, it was noticed in the previous morphological studies^{21,22} that the changes which might occur

TABLE IV CALCULATED CYTOPLASMIC MEMBRANOUS STRUCTURES IN RAT SYMPATHETIC NEURONS

Age	Kind of Animals	Animals No.	Granular Endoplasmic Reticulum (gER)		Golgi Apparatus (Go)	
			No. of Crossings of Sampling Lines with Membrane Profiles of gER	Ratio of Square μ gER to Cubic μ Cytoplasm	No. of Crossings of Sampling Lines with Membrane Profiles of Go	Ratio of Square μ Go to Cubic μ Cytoplasm
40-50 Days	SHR*	147034	1502	2.75	526	0.94
		147035	1508	3.59	538	1.28
		147039	1216	2.41	716	1.33
		157001	1342	3.43	538	1.38
		157002	1116	3.17	413	1.28
		157003	1210	3.08	482	1.23
	MV $\pm$ SE**			3.20 $\pm$ 0.19"		1.23 $\pm$ 0.08'
	Control	724	624	1.93	302	0.92
		725	816	2.09	312	0.81
		728	824	2.01	354	0.86
		729	662	2.36	383	1.00
		730	1118	2.14	528	1.00
		731	626	1.97	324	1.02
	MV $\pm$ SE**			2.09 $\pm$ 0.06"		0.96 $\pm$ 0.08'
4-5 Months	SHR*	147024	1545	2.25	444	0.65
		147025	1704	2.80	667	1.02
		147025	1406	2.96	624	1.31
		147026	838	2.74	351	1.15
		147031	866	2.86	316	1.03
		147032	906	2.30	396	1.00
	MV $\pm$ SE**			2.61 $\pm$ 0.13'		1.03 $\pm$ 0.10
	Control	701	838	1.55	448	0.87
		702	460	1.88	278	1.13
		703	602	1.21	360	0.63
		704	902	2.52	322	0.91
		707	1157	2.30	532	0.98
		711	656	2.40	225	0.82
	MV $\pm$ SE**			1.98 $\pm$ 0.21'		0.89 $\pm$ 0.08
Over 1 Year	SHR*	147005	1126	3.06	521	1.41
		147009	1718	2.92	718	1.23
		147011	892	2.92	465	0.75
		147016	1321	2.48	582	1.10
		147020	1218	2.96	698	1.62
		147023	1612	2.72	718	1.21
	MV $\pm$ SE**			2.85 $\pm$ 0.15"		1.23 $\pm$ 0.13
	Control	645	916	2.20	418	1.00
		646	1316	2.41	622	1.15
		647	910	2.46	364	0.99
		648	526	1.84	296	1.05
		649	788	1.76	496	1.12
		650	968	1.84	478	0.92
	MV $\pm$ SE**			2.09 $\pm$ 0.15"		1.00 $\pm$ 0.03

* Spontaneously Hypertensive Rats

** Mean Value $\pm$ Standard Deviation

' Statistically Significant (0.05 $>$ P $>$ 0.01)

" Statistically Significant (0.01 $>$ P)

in the sympathetic neurons of S.H. rats were relatively slight in degree, comparing them with such severe ones as those which resulted from artificially induced functional disturbances. Except for some cases, significant changes in the sympathetic neurons of S.H. rats could not be recognized apparently, but disclosed by some totalizing numerical analyses. At the same time, the aim was to examine whether any difference could be found between entire functional state of the sympathetic neurons in S.H. rats and in control rats, and it is a fact that the relationship between the detailed submicroscopic structure of neurons and their functional activity has been not invariably established up to the present. Consequently, the gross but generalized observations or summarized quantitative estimations on the changes of cellular constituents may be suitable for the present purpose.

Electron microscopic findings on cellular ultrastructure of the superior cervical sympathetic ganglion in normal Wistar rats did not differ from the common submicroscopic features of sympathetic neurons described already by several authors^{1, 5, 6, 10, 11, 42, 43, 47, 51, 52}, or especially from those of rats presented by FORSSMANN¹³, but were confirmed to coincide with their observations; the details are beyond this discussion. There was also a relatively small change which might result from aging process in the animals. Only lysosomal particles increased in the older animals. This might be related to lipofuscin pigment deposition in the aged^{27, 30}. However, it was likely that the regressive changes occurred to a comparatively small extent at least in the rats before one year of age.

By the quantitative analyses of submicroscopic structure of neurons, as well as by some general observations, certain differences were found between the findings in S.H. rats and those in normotensive controls. To evaluate these findings, some outlined considerations will be made on the functional significance of cell organelles, not being a leading purpose in this study.

A most conspicuous feature in the sympathetic neurons of S.H. rats was abundance in the granular endoplasmic reticulum as well as the

free ribosomes. As generally accepted, the ribosomes in granular endoplasmic reticulum are basic materials directly involved in the protein synthesis. Extensive consideration on the ribosomal mechanism of protein synthesis is beyond the scope of this study. Also in neurons, fundamental significance of this structure may be identical. It has been proved that the ribonucleic acid may act as a template for protein synthesis, the process involving reciprocal control with nuclear desoxyribonucleic acid, and the specificity of proteins synthesized in ribosomes comes from the messenger RNA in the nucleus which receives genetic information from DNA². Direct evidence that protein synthesis occurs in Nissl materials has been recently provided autoradiographically at an electron microscopic level⁹. Some of these proteins may be concerned with membrane synthesis or repair, general metabolic enzymes and special neuronal protein such as the enzymes involved in producing synaptic transmitter, e.g. choline acetylase, or transmitter deactivator like choline esterase¹⁵. Some authors found the acetylcholine esterase activity in the cytoplasm of ganglionic neurons and the correspondence in distribution of reaction product with that of Nissl substance^{14, 32}. Acid phosphatase activity in granular endoplasmic reticulum of some kinds of neurons was also reported²⁹. These facts indicate the significance of granular endoplasmic reticulum on neuronal protein metabolism, and the increased granular endoplasmic reticulum in the ganglionic neurons may suggest the augmented protein metabolism level or, in consequence, neuronal activity.

That the most important mitochondrial function is energy production and transfer through respiration is well established²⁶. High oxidative enzyme activities have been proved in mitochondria; complete enzyme system of Krebs cycle, electron transporting system and oxidative phosphorylation system are contained. Besides, certain evidences which suggest the presence of protein or fatty acid synthesis in mitochondria have been described²⁴. In term of general metabolic activity, functional meaning of the mitochondria in neurons are quite identical to those in general¹⁷. Morphological changes of mitochondria in various physiologi-

cal and pathological states have been extensively studied⁴⁵. Briefly speaking, mitochondrial hyperplasia occurs according to increase of cellular activities⁴. Swelling of mitochondria has been also observed under certain conditions. For example, hypoxia of rabbit cerebellum was accompanied by mitochondrial swelling and cristolysis in neurons²⁵. In the sympathetic neurons pathological states as axon reaction induce mitochondrial swelling¹⁶. Especially in regard to the findings in this study, a small amplitude swelling which occurs physiologically or actively may be important. PACKER stated that swelling of mitochondria is related to the activity of electron transporting^{36,37}.

As to the Golgi apparatus in neurons, the function has not yet been established. Generally, this structure has been considered to be concerned with condensation for export of proteins synthesized at the ribosomes^{3,44,48}, and formation of membrane⁵⁰, as well as synthesis or concentration of complex polysaccharide^{35,41}. Golgi apparatus of neurons apparently resembles that of secretory cells in acquiring newly synthesized protein from endoplasmic reticulum²⁹. EVANS and GRAY¹² found that numerous units of the Golgi apparatus occurred throughout the cytoplasm in the regenerating phase after chromatolysis of neurons.

Functional aspects of lysosomes, whose biochemical concept of DE DUVE⁷ is at present a broad biological concept, have not been clarified in neuron. In general, these structures are accepted as the collection of enzymes that, if released, can alter the cell and its milieu, and a part of an effective device which allows the cell to catalyze organic matter incorporated in bulk, or segregated upon focal autolysis³⁹. Few biological data are available on lysosomes from nervous tissue, but lysosomal nature of the dense bodies in neuron has been established^{18,31}. Novikoff discussed recently on the lysosomes in neurons³⁰. The acid hydrolases produced at endoplasmic reticulum are distributed through Golgi saccles or vesicles (as primary lysosome) to secondary lysosomes such as dense bodies, pinocytotic vesicles, multivesicular bodies or autophagic vacuoles. Intracellular digestion also occurs in endoplasmic reticulum. These suggest the relationship of lysosomes with cel-

lular metabolic activities, though being rather catabolic in character.

Considering the above-mentioned functional roles of various cell organelles in neuron, the following interpretations may be possible: The predominance in the granular endoplasmic reticulum or mitochondria in sympathetic neurons of the S. H. rats of all ages is considered as showing a higher metabolic level or cellular activity as compared with the normotensive rats. Moreover, the neuronal Golgi apparatus was well developed in the S. H. rats of younger ages, and this may suggest that the sympathetic neuronal activity is augmented especially in the rats whose pressor mechanism may more actively function. That the lysosomal fraction was also dominant in the S. H. rats is thought to indicate increased metabolic activity of sympathetic neurons from the catabolic aspect.

In conclusion, increase of sympathetic neuronal activity in the S. H. rats, which was already assumed through some morphological examinations, might be confirmed with electron microscopic observations on the sympathetic neurons. However, the finer observations on structural and functional organization of individual ultrastructures of sympathetic neurons, or especially on synaptic areas and related structures, are the problems of further investigation.

SUMMARY

1. Electron microscopic observation of the neurons in superior cervical sympathetic ganglion was made on a total of 18 spontaneously hypertensive rats (OKAMOTO and AOKI), and 18 normotensive rats of Wistar strain. Evaluation of the findings was performed by quantitative estimation of cytoplasmic ultrastructure with an aid of linear analytical procedures.

2. The ultrastructural organization of neurons in normal Wistar rats coincided with those of rats already described by certain authors.

3. The neurons of the S. H. rats showed submicroscopic features which did not differ in a basic way from those of normotensive control rats. By quantitative analyses of these neurons, however, the following disparate findings were noticed.

- (1) Granular endoplasmic reticulum was

more abundant in the neurons of spontaneously hypertensive rats of all ages as compared with those of normotensive controls. Free ribosomes also showed a similar tendency.

(2) Total mitochondrial volume was greater in the neurons of spontaneously hypertensive rats than in those of normotensives through all ages; no remarkable morphological abnormality of mitochondria was seen except for a slight swelling or cristolysis found in some of them.

(3) Only in the spontaneously hypertensive rats of prehypertensive stage was the Golgi apparatus better developed than in the controls, though the same tendency was suggested through all ages.

(4) More lysosomes were found in the spontaneously hypertensive rats than in the normotensives, being obvious especially in the older ones.

5. From these findings the possibility of higher metabolic level in the sympathetic neurons and, in consequence, of the increase in sympathetic neuronal activity in the spontaneously hypertensive rats were discussed in relation to functional significance of neuronal ultrastructure.

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