Enzyme-histochemical Studies on the Brain Stem of Spontaneously Hypertensive Rats*

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IN THE spontaneously hypertensive rats (abbreviated hereafter as S. H. rats) separated successfully by OKAMOTO and AOKI and maintained in our laboratory^{74, 75, 78}, accumulated observations have revealed the possibility of pathogenetical participation of both the endocrine organs and the autonomic nervous system, as already reported^{3-6, 62, 74, 76, 77, 93}. It is well-known that such functions are controlled by the hypothalamus in some way, and from this point of view, this area was examined precisely in spontaneously hypertensive rats with histochemical methods by our colleagues⁷⁹. The results of these studies showed the possible hyperfunction of hypothalamoadrenocortical and thyroid axis as well as a slight overactivity of hypothalamic sympathetic and parasympathetic zones, and seemed to ascertain the above-mentioned interpretation.

It has been stated, however, that the other portions of the central nervous system also participate in various autonomic somatovisceral functions including circulatory regulation, though their contribution may vary in their extent, and a considerable amount of descriptions^{1, 2, 7-9, 12, 22-25, 29, 30, 37, 40, 43, 44, 48-50, 56-58, 60, 64, 66, 69, 71, 73, 81, 82, 84, 87, 95-97} are available as to the localizations of various autonomic functions in brain stem. From these observations some functional "centers" such as the cardiovascular or respiratory center have been postulated^{1, 7, 12, 29, 69, 72, 81, 82, 84, 87}, and various points which manifest pecu-

liar autonomic responses have been found^{23-25, 37, 49, 50, 56, 57, 64, 95-97}. Despite these contributory works it is a fact that, up to the present time, little has been known about the precise functional organization in brain stem because of its architectural complexity represented by reticular formation and probable functional overlapping in this brain area^{17, 86}. But, considering its functional importance on cardiovascular performance as well as other autonomic functions, some approach may reasonably have to be made to the brain stem in studying the hypertensive subjects.

In the present study, we examined some nuclei of the brain stem of spontaneously hypertensive rats applying the enzyme-histochemical methods which, as already pointed out⁷⁹, seem to be most suitable procedures in exploring the cellular functions morphologically.

MATERIALS

Enzyme-histochemical studies were carried out on the brain stem of the following materials (Table I).

1. 11 spontaneously hypertensive male rats consisted of F₁₁ to F₁₂ rats. They were divided into two groups according to survival duration; i. e. those sacrificed in 40 to 60 days after birth (Stage I) and 3.5 to 4 months after birth (Stage II). The methods of production of S. H. rats were as previously reported^{75, 78}.

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2. As for controls, a total of 11 Wistar strain rats were used. They consisted of those supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University, and their offsprings bred in our laboratory. From among them only normotensive rats (those with blood pressure below 149 mm. Hg) of almost the same age and body weight as individual S. H. rats were selected and used for control materials.

The case numbers of rats used in this study are as follows: The group of rats in Stage I consisted of 6 S. H. rats* (128074, 128075, 128085, 128086, 128092, 128093) and 6 controls (351, 352, Tm97, Tm99, 98, Y1); that in Stage II, of 5 S. H. rats* (111901, 111913, 116005, 116006, 125002) and 5 controls (702, 708, 353, 354, 2). The body weight and blood pressure level of these animals at sacrifice were as shown in Table I.

All these animals were kept in the animal room with complete air conditioning (room temperature 22°–24°C, humidity 50–60 per cent) and artificial lighting. They were fed on

Oriental's stock chow diet (Oriental NMF, Oriental Yeast Co., Japan) or mixed diet of boiled Manitoba wheat and cooked dried sardines, seasoned with a little salt (containing 0.3 per cent sodium), and green vegetables. Tap water was given for drink ad libitum.

METHODS

Blood pressure and body weight of the animals were measured once weekly beginning at 5 weeks of age all through life. For blood pressure determination the modified tail-water plethysmographic method⁷⁵ was employed.

The methods for enzyme-histochemical examinations on the brain stem of animals were principally based on those employed for histochemical studies on the hypothalamus, which have been previously reported from our laboratory⁷⁹. All animals were killed by decapitation early in the morning after 24 hours of isolation in individual cages. Immediately after sacrifice of the animal, the brain was extirpated rapidly, and the brain stem (from the level of rostral border of midbrain to the pyramidal decussation of medulla oblongata) was cut out, frozen indirectly by dry ice-aceton-hexan method⁹³ and stored in a deep

TABLE I MATERIALS SUBJECTED TO ENZYME-HISTOCHEMICAL STUDIES ON THE BRAIN STEM OF ALBINO RATS

Experimental Stage*	Case No. of SHR**	Age***	Body Weight at Sacrifice (gm)	Final Systolic Blood Pressure (mm.Hg)	Case No. of Normotensive Controls	Age***	Body Weight at Sacrifice (gm)	Final Systolic Blood Pressure (mm.Hg)
I	128074	56D	160	144	351	51D	120	112
	128075	56D	170	144	352	51D	120	108
	128085	45D	120	130	Tm97	43D	105	110
	128086	45D	105	136	Tm99	42D	110	105
	128092	48D	170	140	98	50D	150	110
	128093	48D	145	132	Y1	57D	145	112
	MV±SD****		145±27.2	138±6.0	MV±SD****		125±18.4	110±2.7
II	111901	3.5M	260	195	702	3.5M	275	132
	111913	3.5M	245	210	708	3.5M	250	120
	116005	4M	265	182	353	4M	280	118
	116006	4M	280	180	354	4M	260	120
	125002	4M	276	200	2	4M	310	124
	MV±SD****		265±13.9	193±12.5	MV±SD****		275±22.9	123±5.6

* For designation, see text.

** Spontaneously hypertensive rats.

*** D: Days after birth; M: Months after birth.

**** Mean value ± standard deviation.

* The S. H. rats were obtained by the following matings: 128074 ♂ and 128075 ♀ (110049 ♂ × 110089 ♀); 128085 ♂ and 128086 ♂ (118004 ♂ (obtained by 10169 ♂ × 10178 ♀) × 110119 ♀); 128092 ♂ and 128093 ♂ (110049 ♂ × 118051 ♀ (obtained by 10087 ♂ × 10172 ♀)); 125002 ♂ (110113 ♂ × 110115 ♀); 111901 ♂ (T10202 ♂ × T10204 ♀); 111913 ♂ (T10202 ♂ × T10207 ♀). The details of the source of these parent rats, as well as 116005 ♂ and 116006 ♂, were previously reported^{75, 78}.

freezer. Serial sections were cut at 30 micron from the prepared frozen blocks in a cryostat (Dittes-Duspiva, Germany) which was maintained at -20°C , and attached to a thinner slide glass by momentary thawing by the heat of fingers. At that time, arrangement of sections was performed by taking every fourth section ordinarily to make four groups of slides from one series of sections.

Four kinds of enzymes, i.e. acid phosphatase (ACPase), diphosphopyridine nucleotide diaphorase (DPND), monoamine oxidase (MAO) and acetylcholine esterase (AChE), were examined on these unfixed frozen sections by the following histochemical methods.

ACPase: Metal-salt (Pb) method (GOMORI^{24,25}, slightly modified by TAKAMATSU^{80,93}).

DPND: Tetrazolium method (BURSTONE²⁰).

MAO: Tetrazolium method (GLENNER et al.³³).

AChE: Acetylthiocholine method (GOMORI²⁵).

10^{-6}M di-isopropylfluorophosphate in final concentration was used as an inhibitor in incubating medium^{10,45}.

The details of the staining methods were performed after the manner of those described in the preceding papers^{79,93}. Throughout all these histochemical procedures great caution was paid to treat materials from experimental groups and those from corresponding control groups simultaneously and under strictly identical histochemical conditions.

Evaluation of findings obtained by the above-mentioned methods was made as follows. Identification of the cytoarchitectures of brain stem was mainly placed on the VALVERVE's studies⁹⁴, supplemented by other descriptions^{46,98}, and the nomenclatures of brain stem structures were abbreviated in this study as follows:

Rd : Subnucleus reticularis dorsalis medullae oblongatae
Rv : Subnucleus reticularis ventralis medullae oblongatae
Nrl : Nucleus reticularis lateralis
Ndm : Nucleus originalis dorsalis nervi vagi
Nrp : Nucleus reticularis paramedianus
Rpc : Nucleus reticularis parvocellularis
Rgc : Nucleus reticularis gigantocellularis
Nrt : Nucleus reticularis tegmenti pontis (BECHTEREW)
Nrpc : Nucleus reticularis pontis caudalis
Nrpo : Nucleus reticularis pontis oralis
EW : Nucleus accessorius caudalis (EDINGER-WESTPHAL)

Comparison of the results of experimental groups with those of controls was carried out quite objectively with a light microscope on 11 nuclei of the brain stem. Several doctors were engaged in judging the results of the experimental groups, which were designated as "increased" or "decreased" enzyme activity, or "no noticeable change" in comparison with the corresponding controls, with a cautious manner to avoid any prejudice.

RESULTS

The findings of the S. H. rats were always compared with those from the normotensive controls of almost the same body weight and survival duration.

Boby weight and blood pressure (Table I): There was no meaningful difference between mean body weight of the S. H. rats and that of the controls in both Stages I and II, though the

TABLE II DISTRIBUTION OF ENZYME ACTIVITIES IN THE BRAIN STEM NUCLEI OF NORMOTENSIVE CONTROL RATS

Experimental Stage	I				II			
	ACPase	DPND	MAO	AChE	ACPase	DPND	MAO	AChE
Rd	++	+	+	±	+	++	+	±
Rv	++	+	+	±	+	+	+	±
Nrl	+++	+++	+	++	+++	+++	+	++
Ndm	+++	+++	++	++	+++	+++	++	++
Nrp	++	++	+	±	+	+	+	±
Rpc	+	++	+	±	+	±	+	±
Rgc	+++	+++	+	+	+++	++	+	+
Nrt	+++	+++	+	±	+++	++	+	±
Nrpc	+++	+++	+	+	+++	+++	+	+
Nrpo	+++	+++	±	±	+++	++	±	±
EW	++	++	+	++	++	++	+	++

Grades of staining

+++ : Most intense staining

++ : Moderate staining

++ : Intense staining

± : Slight staining

For abbreviations of enzymes and nuclei, see text.

average value of S. H. rats seemed to be a little higher than the controls in Stage I.

Mean systolic blood pressure level of the S. H. rats in Stage I was 138 ± 6.0 mm. Hg and still remained in normotensive range (blood pressure above 150 mm. Hg was considered as hypertension⁷⁵), but was significantly higher than that of the controls (110 ± 2.7 mm. Hg). The S. H. rats in Stage II showed much higher blood pressure (193 ± 12.5 mm. Hg in average) which exceeded that of the controls by about 70 mm. Hg.

Enzyme activities in the brain stem of the normotensive rats: Four kinds of enzymes (ACPase, DPND, MAO, and AchE) were studied histochemically by the above-mentioned methods, and the activities of these enzymes in the brain stem nuclei of normotensive control rats, which may be considered to show the normal distribution of enzyme activities, are illustrated in Table II. Throughout all brain stem nuclei examined in this study, the activities of these four enzymes in each nucleus showed almost equal intensity in young (Stage I) and adult (Stage II) rats except for minute differences in several nuclei, which may be not so meaningful at present, because these two control groups

were not treated under the strictly same histochemical conditions.

In the case of ACPase the perikaryon of nerve cells, as well as some nerve fibers, were stained clearly in contrast with the residual brain substances. Most intense staining was found in the nuclei such as Nrl, Ndm, Rgc, Nrt, Nrpc and Nrpo, and other nuclei showed moderate or slight staining.

DPND also showed relatively deep staining in the brain stem. Microscopically, however, the detailed presentations of the cytoplasm of nerve cells were, in contrast with neuropils, not so clearly demonstrated as in the case of ACPase, especially in the nuclei examined in the present study. Among the brain stem nuclei examined, Nrl, Ndm, Rgc, Nrt and Nrpo revealed fairly intense DPND activity. Only Nrpc showed slight staining in Stage II, though this nucleus was stained intensely in Stage I.

MAO and AchE activities in the brain stem nuclei were generally not so prominently demonstrated as in the case of ACPase or DPND, although the reactions seemed to be relatively intense as compared with the other brain areas such as the cerebral cortex. MAO activity was scarcely localized in the perikaryon of nerve

TABLE III ENZYME-HISTOCHEMICAL FINDINGS ON THE BRAIN STEM OF SPONTANEOUSLY HYPERTENSIVE RATS IN COMPARISON WITH NORMOTENSIVE CONTROL RATS

Experimental Stage	I				II			
Enzyme	ACPase	DPND	MAO	AchE	ACPase	DPND	MAO	AchE
Nucleus								
Rd	—	↑ (1/4)	↑ (1/6)	—	↑ (1/5)	—	—	—
Rv	—	—	—	—	—	—	—	—
Nrl	—	—	—	—	↑ (4/5)	↑ (1/5)	—	—
Ndm	↑ (3/6)	↑ (4/4)	↑ (3/6)	↑ (4/6)	↑ (5/5)	↑ (4/5)	—	↓ (2/5)
Nrp	↑ (2/6)	—	↓ (1/6)	↑ (2/6)	—	—	—	↑ (2/5)
Rpc	↓ (2/6)	—	↓ (1/6)	↓ (1/6)	—	—	—	—
Rgc	↓ (2/6)	↑ (3/4)	↓ (2/6)	—	↑ (2/5)	—	—	—
Nrt	↑ (3/6)	↓ (2/4)	↑ (2/6)	—	—	↑ (1/5)	—	↓ (1/5)
Nrpc	—	—	—	—	—	↑ (1/5)	↓ (1/5)	—
Nrpo	—	↑ (3/4)	—	↑ (1/6)	↓ (2/5)	—	↑ (1/5)	—
EW	↓ (2/6)	—	↑ (4/6)	—	↑ (2/5)	↑ (3/5)	↑ (2/5)	↑ (2/5)

Notes: ↑, ↑ : Increase of enzyme activity. The former shows that the cases with increased activity were found in more than half of cases examined, and the latter, in less than half.

↓, ↓ : Decrease of enzyme activity. The former shows that the cases with decreased activity were found in more than half of cases examined, and the latter, in less than half.

— : No remarkable change.

() : The numbers parenthesized express the number of cases of the indicating finding (numerator) and total number of cases examined (denominator).

For abbreviations, see text.

cells, but diffusely recognized in the neuropils, and occasionally the nerve fibers were clearly stained. In the brain stem Ndm showed moderately intense reaction of MAO as compared with the other nuclei which revealed only slight staining. AchE activity was more confined to nerve cell bodies than in the case of MAO, but the neuropils were also stained to a varied extent in some brain stem areas. Nrl, Ndm and EW showed relatively distinct AchE activity among the nuclei examined.

Enzyme activities in the brain stem of the spontaneously hypertensive rats: The findings from the S. H. rats, which were obtained by the comparison with each corresponding normotensive control rats, were considered to be relevantly positive when changes of the same direction (increased or decreased activity) were observed

on more than one half of cases examined, and the results were as illustrated in Table III.

1. ACPase Case numbers of the S. H. rats were as follows: 128074, 128075, 128085, 128086, 128092 and 128093 (Stage I); 111901, 111913, 116005, 116006 and 125002 (Stage II).

Increased ACPase activity was observed in Ndm (3 cases) in Stage I (Fig. 1), Nrl (4 cases) and Ndm (5 cases) in Stage II (Fig. 2). Activity tended to decrease in Nrt (3 cases) in Stage I.

2. DPND Case numbers: 128085, 128086, 128092 and 128093 (Stage I); 111901, 111913, 116005, 116006 and 125002 (Stage II).

Increase of DPND activity was observed in Ndm (4 cases) (Fig. 3), Rgc (3 cases) and Nrpo (3 cases) in Stage I, and Ndm (4 cases) (Fig. 4)

Fig. 1. ACPase activity in the dorsal vagal nucleus. (By metal-salt method). ($\times 400$).
Left: A normotensive rat (No. 98, male, 50 days old).
Right: A S. H. rat (No. 128093, male, 48 days old).
More intense reaction in the cytoplasm is observed in the S. H. rat as compared with the normotensive rat.

Fig. 2. ACPase activity in the dorsal vagal nucleus. (By metal-salt method). ($\times 400$).
Left: A normotensive rat (No. 708, male, 3.5 months old).
Right: A S. H. rat (No. 111901, male, 3.5 months old).
Reaction can be more prominently observed in the S. H. rat than in the normotensive rat.

and EW (3 cases) in Stage II. Only Nrt in Stage I showed decreasing tendency of activity (2 cases).

3. MAO Case numbers: 128074, 128075, 128085, 128086, 128092 and 128093 (Stage I); 111901, 111913, 116005, 116006 and 125002 (Stage II).

Enhancement of MAO activity was observed in Ndm (3 cases) (Fig. 5) and EW (4 cases) in Stage I. No confirming finding could be obtained throughout all nuclei in Stage II.

4. AchE Case numbers: 128074, 128075, 128085, 128086, 128092 and 128093 (Stage I); 111901, 111913, 116005, 116006 and 125002 (Stage II).

Only Ndm in Stage I showed increased AchE activity (4 cases) (Fig. 6) in this study. No other noticeable change could be found in both Stages I and II.

DISCUSSION

Similar to what was reported previously from this laboratory^{75,78}, the S. H. rats subjected to this study also showed higher blood pressure level than that of normotensive control rats. The mean blood pressure of S. H. rats at 40 to 60 days of age (Stage I) was still in normotensive range (so, this stage was designated as "pre-hypertensive stage" in the preceding paper⁷⁸), but it was significantly higher than that of controls even at this stage. In the S. H. rats at 3.5 to 4 months after birth (Stage II) blood pressure reached a considerably high level, exceeding by far that of the controls, and this stage corresponds principally to "initial stage of hypertension" in our designation⁷⁸.

Body weight of the S. H. rats both in Stage I and Stage II revealed no significant difference

Fig. 3. DPND activity in the dorsal vagal nucleus. (By tetrazolium method). ($\times 400$).
Left: A normotensive rat (No. 99, male, 50 days old).
Right: A S. H. rat (No. 128092, male, 48 days old).
Reaction is relatively well-defined to the perikaryon but the neuropils are also stained to some extent. Enhancement of activity in the S. H. rat can be seen.

Fig. 4. DPND activity in the dorsal vagal nucleus. (By tetrazolium method). ($\times 400$).
Left: A normotensive rat (No. 353, male, 4 months old).
Right: A S. H. rat (No. 116005, male, 4 months old).
Increased activity in the S. H. rat is observed.

from that of the normotensive controls also, as has been already stated^{75,78}.

In evaluating the results obtained by the present study, the first problem of importance is the relationship between enzyme activities in nervous tissue and neuronal activity. As to this point, however, detailed discussion was made in the preceding paper⁷⁹ and, at present, it will be unnecessary for us to repeat the identical interpretations. In brief, ACPase is thought to be one of the favourable enzymes for histochemical examinations of nerve cell functioning by the fact that its activity moves according to variously induced functional changes of neurons^{90,91}, or that ACPase seems to be, at least in some part, related to the function of intraneuronal nucleoproteins^{11,15,21,36,41,42,51,52,54,55,85}, and, at the same time, owing to the clarity of this enzyme

in displaying the chemoarchitectonics of nervous system¹³ as well. DPND, which has been considered as mitochondrial electron transporting enzyme¹³, may also play some role in neuronal activity⁴⁷, especially in relation to energy metabolism. As to MAO and AchE, these enzymes may be considered to play a significant role in controlling the nervous functions from the point of view that they are involved in the metabolism of biogenic amines and acetylcholine in the central nervous system, which may be, more or less, concerned with the nervous impulse transmission, or other certain functions^{16,18,26,27,28,31,39,63,68,70,88,89}. Particularly concerning MAO, it is said that the activity of this enzyme is fairly specific to the autonomic regions of the brain³⁸. It can be said, therefore, that changes in activities of these enzymes may

Fig. 5. MAO activity in the dorsal vagal nucleus. (By tetrazolium method). ($\times 400$).
Left: A normotensive rat (No. Tm 99, male, 42 days old).
Right: A S. H. rat (No. 128086, male, 45 days old).
Activity is hardly defined to the cytoplasm but diffusely observed in the neuropils. More intense reaction is found in the S. H. rat.

Fig. 6. AchE activity in the dorsal vagal nucleus. (By acetylthiocholine method). ($\times 400$).
Left: A normotensive rat (No. Tm 97, male, 45 days old).
Right: A S. H. rat (No. 128085, male, 45 days old).
Both the cytoplasm and the neuropils are stained but not so sharply defined. The S. H. rat shows more enhanced activity than the normotensive rat.

reflect certain, if not all, functional states of the neurons or other nervous structures, though without complete authenticity.

The second problem is the functional organization of the brain stem, especially concerning the autonomic functions. As regards this question, though a lot of available observations have been made by many authors up to the present time, it seems that no conclusive evidence has yet been found. In connection with blood pressure, or cardiovascular regulation, gross destruction or ablation, and later, stimulation of brain stem led to the assumption of specific vasomotor controlling areas^{1, 2, 7-9, 29, 44, 61, 65, 73, 82, 84, 87}. The present concept on this brain stem cardiovascular center has been largely based on that described by ALEXANDER¹. Though there are some differences as regards the distributions according to individual authors, it has been stated that the pressor area extends widely from the dorsomedial and ventrolateral quadrants of the reticular formation in the rostral end of the medulla oblongata, shifting to central and dorsomedial in the middle portions of the medulla, to the most lateral parts of dorsal and ventral bulbar areas at the caudal level. On the other hand, according to ALEXANDER or some other authors,^{1, 2} the extension of depressor areas seems to be limited fairly exclusively in a medial portion between the inferior oliva and hypoglossal nucleus, while other explorers found a greater dispersal⁷².

Concerning autonomic functions of the brain stem including the above-mentioned cardiovascular performance, extensive experimental studies have been carried out since CLAUDE BERNARD found "diabète artificiel" in rabbits¹⁴, and attempts to clarify the question of various autonomic centers, or to find out relationships between physiological autonomic responses and anatomical organization of the brain stem have been made by many investigators^{23-25, 37, 49, 50, 56, 57, 64, 95-97}. Up to the present time, however, no one has succeeded in presenting a precise description on this subject. Essential as it is, it is not the main purpose to discuss the details, and subject will be confined to only an outline, especially centering on brain stem reticular formation for the present. Roughly speaking, the sympathetic zones, or the portions which can

elicit sympathetic responses in brain stem, have been found in central gray matter of mesencephalon and rhombencephalon, dorsomedial and parts of ventrolateral pontine reticular formation, dorsal portion of upper myelencephalic reticular formation, ventral parts of lower myelencephalic reticular formation etc.; parasympathetic responses have been observed in some dorsal parts of pons, ventral part of upper myelencephalic reticular formation, dorsomedial portion of lower myelencephalic reticular formation, dorsal motor nucleus of vagus and so on.

In the preset study we examined histochemically the enzyme activities of some reticular or other nuclei in the brain stem of the S. H. rats. As was expected, there was considerable dispersal in the findings and evaluation of the results was somewhat difficult, probably owing to the anatomical as well as functional complexity of this region of the brain.

Among the nuclei investigated, fairly definite findings were observed in the dorsal motor nucleus of vagus (Ndm), which showed increased activity of all enzymes examined in Stage I, and of ACPase and DPND in Stage II. Although the functions of Ndm has not yet been established, it is said that this is, at least, one important source of vagal, or cranial parasympathetic preganglionic fibers, and the area including this nucleus is thought to belong to the parasympathetic system^{19, 32, 38, 53, 67, 92}. As regards blood pressure regulation this nucleus seems to be involved in the depressor zone. The important problem concerning the baroreceptor reflex area is not yet thoroughly solved, and no distinct proof that this reflex area is Ndm itself has yet been found^{59, 60, 72, 83}. But depressor centers have little autonomy, and it may be admitted that they are constantly activated by afferent impulses from baroreceptor regions^{72, 83}. Taking these into consideration, the increase of the enzyme activities in Ndm of the S. H. rats may be regarded as showing the enhanced parasympathetic, or other related functions in the brain stem to some extent.

On the nuclei other than Ndm, some positive findings could be seen, though restricted within one or two enzymes. Nrl and Nrpo showed increased ACPase activity in Stage II, and DPND

activity in Stage I, respectively. Rgc revealed increased DPND activity in Stage I, and EW showed increased MAO activity in Stage I and DPND activity in Stage II. In these the former two nuclei are likely to belong to sympathetic system and the latter to parasympathetic system, and these findings may indicate overactive state of respective autonomic regions in the brain stem of the S.H. rats. Reduced ACPase and DPND activities were found only in Nrt in Stage I, which may also belong to parasympathetic zone, but the meaning of this findings is not clear.

As is known, brain stem has a complicated organization, functionally as well as anatomically, represented by important but unsolved mechanism of reticular formation^{17,86}. It is a question whether we can determine any functional zones in brain stem so simply as above-mentioned, but, insofar as the findings of the present study show, it may be suggestive that the autonomic regions in the brain stem of the S.H. rats is, if not all, in overactive state, which was most distinctly observed in Ndm in this study. Etiological participation of autonomic nervous system in S.H. rats has already been pointed out^{62,76,77,79,93}, and the results of the present study may be compatible with this interpretation. However, the questions as to the primary site of this autonomic overactivity or the extent of participation of brain stem in blood pressure control in S.H. rats is a problem for further investigation.

SUMMARY

1. Enzyme-histochemical study was performed on the brain stem of spontaneously hypertensive rats (OKAMOTO and AOKI). Four kinds of enzyme activity (acid phosphatase, diphosphopyridine nucleotide diaphorase, monoamine oxidase and acetylcholine esterase) were examined on 11 nuclei of brain stem of a total of 11 spontaneously hypertensive rats and 11 normotensive control rats of Wistar strain, which were subdivided into two groups according to survival duration (40–60 days after birth (Stage I) and 3.5–4 months after birth (Stage II)). Each positive finding in spontaneously hypertensive rats was obtained by comparing it to that of normotensive controls.

2. Mean systolic blood pressure of spontaneously hypertensive rats was significantly higher than that of controls, despite that, it was still in normotensive range in Stage I. Mean body weight did not show any significant difference between spontaneously hypertensive rats and control rats.

3. As to the normal distribution of enzyme activities in the brain stem, an outline of the reactions observed in the brain stem nuclei of normotensive control rats was described.

4. Comparing the results from spontaneously hypertensive rats with those of controls the following findings were obtained. Increased enzyme activities were found in Nucl. originis dorsalis nervi vagi (acid phosphatase, diphosphopyridine nucleotide diaphorase, monoamine oxidase and acetylcholine esterase in Stage I; acid phosphatase and diphosphopyridine nucleotide diaphorase in Stage II), Nucl. reticularis lateralis (acid phosphatase in Stage II), Nucl. reticularis pontis oralis (diphosphopyridine nucleotide diaphorase in Stage I), Nucl. reticularis gigantocellularis (diphosphopyridine nucleotide diaphorase in Stage I), Edinger-Westphal's nucleus (monoamine oxidase in Stage I and diphosphopyridine nucleotide diaphorase in Stage II). Decreased activity was observed only in Nucl. reticularis tegmenti (acid phosphatase and diphosphopyridine nucleotide diaphorase in Stage I).

5. From the above-mentioned findings it was assumed that the autonomic nervous system in the brain stem of the spontaneously hypertensive rats may be in overactive state, being particularly distinct in the parasympathetic zones centering around the dorsal vagal nucleus in this study, though detailed etiological participation of this area could not be clarified.

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