On the Noradrenaline Reaction of the Adrenal Medulla in Experimental Hypertensive Rats, Especially in Spontaneously Hypertensive Rats

I. Histochemical and Histometrical Studies on the Noradrenaline Storing Cell Islets of the Adrenal Medulla in Various Experimental Hypertensive Rats*

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THE STUDY on the relation between adrenal medulla, a productive source of pressor amine, and hypertension was first made in 1894 by OLIVER and SCHÄFER¹ who found that intravenous administration of adrenal extracts to animals was followed by a pressor response. And, since it was reported by NEUSSER² that the oversecretion of this material caused hypertension, the relation between hypertension and catecholamine, especially noradrenaline, has attracted the attention of researchers. Many studies have been made up until today on the secretion and metabolism of noradrenaline and sensitivity of vascular wall to noradrenaline, but it seems to be unreasonable to try to find the cause of hypertension in the oversecretion of noradrenaline only, except for the case of pheochromocytoma.

In 1962 and 1963, OKAMOTO and AOKI reported on a colony of hypertensive rats which were called spontaneously hypertensive rats^{3,4}. These rats, different from other experimental hypertensive rats, show 100 per cent incidence of spontaneous occurrence of hypertension and it resembles the essential hypertension in that no remarkable change is found in kidney and cardiovascular system before the development of hypertension^{4,5}. And many studies on the endocrine^{6,7,8,9} and the cardiovascular system in these rats have been made, and from the result, it has been suggested that spontaneously

hypertensive rats show a slight, but sure increase of the function of adeno-hypophyseal-adrenocortical system and adeno-hypophyseal-thyroid system before the development of hypertension. Recently, it has been reported by Tabei¹⁰ that the area of noradrenaline storing cell islets in the adrenal medulla of these rats increased and that noradrenaline reaction in the adrenal medulla of these rats is stronger than that of control rats, while it was also reported by NASHIMOTO¹¹ that renal hypertensive dogs show an increase of noradrenaline reaction in the adrenal medulla through iodate reaction.

The author has attempted to make histochemical and histometrical studies on noradrenaline storing cell islets in the adrenal medulla of spontaneously hypertensive rats, renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats in order to know more exactly the relation between hypertension and adrenal medulla and to find whether there is any difference in findings on adrenal medulla of spontaneously hypertensive rats and those of other hypertensive rats.

MATERIALS

Both Wistar strain rats supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University and some of their offsprings bred at our Department of Pathology were used. Among the offsprings, some of those with blood pressure below 149 mmHg were used as control rats for the com-

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(Received for Publication, November 7, 1967)

* This study was supported in part by a grant (HE 10762) from the Heart Institute of the National Institute of Health, U.S.A. and in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Japan, and the Chiyoda Mutual Life's Subsidiary Fund for Social Welfare Works.

parison of various other hypertensive rats, the rest having been used for the production of various experimental hypertensive rats.

The following are the methods of production of various hypertensive rats.

(1) Renal hypertensive rats

Renal infarction hypertension was induced by ligation of the bilateral posterior branches of the main renal artery of male rats, 40 to 50 days after birth, weighing 100 to 130 grams, in accordance with the LOOMIS's method¹².

(2) DCA hypertensive rats

50 mg. of DCA in a pellet was implanted into the thigh muscles of male rats, 30 to 40 days after birth, weighing 100 to 140 grams, and 1 per cent salt water was given for drinking in accordance with SELYE's method¹³.

(3) Neurogenic hypertensive rats

In accordance with the method of ROSENFELD¹⁴, TAYLER¹⁵ and TANABE¹⁶, ligated one side internal carotid artery and sinus nerve of male rats, 50 days after birth, weighing 100 to 150 grams, were cut and 10 days later the same operation on the opposite side was made.

The above operations were made under Nembutal anesthesia (Product of Abbott Laboratories; 20 mg./kg. intraperitoneal injection) and for renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats, sham operations were conducted.

(4) Spontaneously hypertensive rats

F₁₂ and F₁₃ rats used in this research were bred from F₁₀ and F₁₁ (110077, 110113, 100017, 100060, 110117, 110112, 100087, 100069, 110049 and 100071) which had been produced in the Department of Pathology¹⁷ from spontaneously hypertensive rats (OKAMOTO and AOKI^{3,4}). The production procedure of spontaneously hypertensive rats has already been reported^{4,17} in detail.

A total of 360 rats was used and they had been raised in an air conditioning room (23±2°C with humidity of about 60 per cent).

These rats were fed on Orientals stock chow diet (NMF and MF) (Oriental Yeast Co., Japan) or boiled Manitoba wheet mixed with cooked dried sardine, and seasoned with a little salt (0.3% Na) and green vegetables. Tap water was given ad libitum except to DCA hypertensive rats.

On the day before killing, each rat was put in a cage and left quiet all through the night, and early the next morning, it was taken out as quietly as possible not to be stimulated and was killed by decapitation within as short a time as possible (hereafter described as minimum stress).

The author observed the noradrenaline storing cell islets of spontaneously hypertensive rats everyday until 16 days after birth, every other day until 20 days after birth, every 5 days until 30 days after

birth and in the following three stages: 40 to 60 days after birth, 5 to 7 months after birth, more than one year after birth: and those of renal hypertensive rats DCA hypertensive rats and neurogenic hypertensive rats in the following four stages: prehypertensive stage (4 to 18 days after operation), early stage (5 to 24 days after operation), middle stage (3 months after operation) and late stage (6 months after operation).

METHODS

General Observations

The blood pressure and body weight were measured once a week all through life after 4 weeks of age. The former was measured indirectly without anesthesia by the modified tail-water plethysmographic method⁴.

Adrenal Weight

The adrenals were taken out immediately after decapitation, and blood, attached adipose tissue and connective tissue were removed as soon as possible. The total weight of both adrenals was weighed on a torsion balance and the value was shown by ratio to body weight.

$$\frac{\text{weight of adrenal (gm)}}{\text{body weight (gm)}} \times 10^5$$

Histochemical and Histometrical Observation of the Adrenal Medulla

(1) COUPLAND et al.'s Glutaraldehyde Osmium Tetroxide Method for Noradrenaline Reaction in the Adrenal Medulla¹⁸

The left adrenals of various hypertensive rats and control rats were removed immediately after decapitation, cut into small pieces of about 5 mm thick, fixed for 24 hours in cold (0–4°C) 6 per cent glutaraldehyde solution buffered to pH 7.3 with 0.1 M phosphate, cut at 15 μ with thermo-electro freezing microtome (Komatsu Electronics Inc., Japan), stained for 2 hours in 1 per cent veronal acetate buffered osmium tetroxide solution (pH 7.3), washed in distilled water and mounted in Apáthy gummy syrup.

(2) Histometrical Observation of Noradrenaline Storing Cell Islets in the Adrenal Medulla

The adrenal slide stained with the above method was projected at the magnification of 310 times on a sheet of graph paper through Ernst Leitz microprojector (Type XIc Xenon, Germany). The total area of adrenal medulla and the area of noradrenaline storing

cell islets were drawn and measured with a planimeter. For each section the dimensional ratio of noradrenaline storing cell islets to the whole adrenal medulla was calculated as follows:

$$\frac{\text{area of noradrenaline storing cell islets}}{\text{area of whole adrenal medulla}} \times 100$$

Three sections of one rat were chosen at random and the area of noradrenaline storing cell islets was determined on the average of these three sections. The author showed the value of increase or decrease in the area of noradrenaline storing cell islets of various hypertensive rats against that of control rats by ratio as follows:

$$\frac{\text{area of noradrenaline storing cell islets in the adrenal medulla of various hypertensive rats (\%)}}{\text{area of noradrenaline storing cell islets in the adrenal medulla of control rat (\%)}}$$

Statistical Analysis

The data thus obtained were statistically analyzed using "t" test, and the data were considered significant when the "p" values were less than 0.01.

RESULTS

(1) Spontaneously Hypertensive Rat Group

The blood pressure, body weight, adrenal weight and the area of noradrenaline storing cell islets in the adrenal medulla are shown in Tables I and II.

Blood Pressure: All spontaneously hypertensive rats showed hypertension (above 150

mmHg) from the 8th week after birth, but control rats remained 106 to 134 mmHg. A significant difference was noted between the two.

Body Weight and Adrenal Weight: There was no significant difference between spontaneously hypertensive rats and control rats in the body weight and the adrenal weight.

Histochemical and Histometrical Findings on the Noradrenaline Storing Cell Islets in the Adrenal Medulla: Noradrenaline storing cell islets in the adrenal medulla of spontaneously hypertensive rats and of control rats were not stained by COUPLAND et al.'s method until 5 days after birth and became stainable on the

6th day after birth, but the intensity of the staining was not strong (Fig. 1 and 2). The area of noradrenaline storing cell islets then was 9.6 per cent in the case of spontaneously hypertensive rats and 8.1 per cent in the case of control rats. The former showed an increase of 1.2 times as large as that of the latter ($0.01 < p < 0.02$). After this period, the area of noradrenaline storing cell islets of spontaneously hypertensive rats showed a gradual increase as follows: 10.9 per cent on the 8th day after birth; 21.7 per cent on the 18th day after birth (Fig. 4); 24.6 per cent on the 30th day after birth; 22.6 per cent on the 40th to 50th day

Fig. 1. Noradrenaline storing cell islets (NSCI) in the adrenal medulla of normotensive rat (NR) at 6th day after birth (Coupland et al.'s method). NSCI stain brown but the intensity of staining is very weak. $\times 40$

Fig. 2. NSCI in the adrenal medulla of spontaneously hypertensive rat (SHR) at 6th day after birth (Coupland et al.'s method). The area of NSCI is about 1.2 times as large as that of NR. $\times 40$

after birth; 25.6 per cent in the 5th month after birth and 26.6 per cent over one year after

TABLE I DIMENSIONAL CHANGES OF THE NORADRENALINE STORING CELL ISLETS
IN THE ADRENAL MEDULLA OF SPONTANEOUSLY HYPERTENSIVE RATS

Days after Birth	No. of Animals	Sex		Mean Body Weight at Sacrifice (gm) M (range)	Adrenal Weight (gm) $\times 10^5$ Body Weight (gm)		Dimension of NSCI $\times 100$ Dimension of Adrenal Medulla			
		♂	♀		M	S. E.	M	S. E.	#	
0	SHR	Not stainable								
1	NR									
5										
6	SHR	5	4	1	11 (9.5-13)	21.6 $\pm$ 1.4	9.6 $\pm$ 0.2**			$\times 1.2$
	NR	5	3	2	10 (10-10.5)	25.7 $\pm$ 2.1	8.1 $\pm$ 0.5			
7	SHR	5	3	2	10 (8-11)	37.5 $\pm$ 4.1	10.9 $\pm$ 0.6***			$\times 1.3$
	NR	5	3	2	12 (11-14)	32.8 $\pm$ 2.5	8.2 $\pm$ 0.5			
8	SHR	5	2	3	15 (14-15)	27.3 $\pm$ 1.9	10.9 $\pm$ 0.6***			$\times 1.3$
	NR	5	3	2	15 (15)	31.9 $\pm$ 1.3	7.6 $\pm$ 0.4			
9	SHR	5	3	2	15 (14-16)	31.9 $\pm$ 0.9	13.7 $\pm$ 0.9***			$\times 1.4$
	NR	5	2	3	16 (15-16)	34.6 $\pm$ 2.3	9.5 $\pm$ 0.6			
10	SHR	5	2	3	16 (15-16)	29.6 $\pm$ 1.9	14.1 $\pm$ 1.1***			$\times 1.5$
	NR	5	2	3	16 (15-18)	29.4 $\pm$ 1.4	9.1 $\pm$ 0.7			
11	SHR	5	3	2	16 (14-17)	32.0 $\pm$ 1.6	14.4 $\pm$ 0.9***			$\times 1.5$
	NR	5	4	1	16 (15-17)	30.9 $\pm$ 1.7	9.9 $\pm$ 1.3			
12	SHR	5	3	2	18 (15-20)	34.6 $\pm$ 1.8	17.7 $\pm$ 0.6***			$\times 1.5$
	NR	5	2	3	16 (15-19)	32.9 $\pm$ 0.4	11.5 $\pm$ 0.5***			
13	SHR	5	3	2	19 (18-21)	36.9 $\pm$ 2.4	21.8 $\pm$ 0.9***			$\times 1.6$
	NR	5	3	2	19 (17-22)	32.9 $\pm$ 2.4	13.8 $\pm$ 0.8			
14	SHR	5	4	1	21 (18-24)	35.9 $\pm$ 2.7	17.1 $\pm$ 0.6***			$\times 1.7$
	NR	5	3	2	23 (20-25)	32.0 $\pm$ 1.3	9.8 $\pm$ 0.6			
15	SHR	5	2	3	24 (20-28)	28.9 $\pm$ 2.6	18.8 $\pm$ 0.4***			$\times 1.8$
	NR	5	2	3	27 (20-30)	35.9 $\pm$ 0.3	10.4 $\pm$ 0.7			
16	SHR	5	3	2	23 (20-27)	36.1 $\pm$ 1.8	19.4 $\pm$ 0.4***			$\times 1.8$
	NR	5	3	2	26 (22-30)	34.6 $\pm$ 1.7	10.9 $\pm$ 0.5			
18	SHR	5	3	2	25 (24-25)	44.4 $\pm$ 1.6	21.7 $\pm$ 1.1***			$\times 2.1$
	NR	5	2	3	24 (20-25)	43.2 $\pm$ 0.8	10.5 $\pm$ 0.9			
20	SHR	5	3	2	27 (25-27)	48.1 $\pm$ 1.7	22.0 $\pm$ 1.8***			$\times 2.1$
	NR	5	2	3	26 (25-27)	46.1 $\pm$ 1.7	10.7 $\pm$ 0.5			
25	SHR	5	3	2	40 (35-47)	47.6 $\pm$ 1.7	20.3 $\pm$ 1.2***			$\times 2.1$
	NR	5	3	2	39 (35-42)	42.8 $\pm$ 1.3	9.7 $\pm$ 0.3			
30	SHR	5	3	2	53 (50-60)	44.4 $\pm$ 1.9	24.6 $\pm$ 1.0***			$\times 2.0$
	NR	5	3	2	50 (45-55)	44.4 $\pm$ 3.3	12.7 $\pm$ 1.0			

NSCI: Noradrenaline storing cell islets M: Mean S. E.: Standard error

SHR: Spontaneously hypertensive rats NR: Normotensive rats

Significant differences compared with values of normotensive group: * $0.01 < p < 0.025$ ** $p < 0.01$

#: The dimensional ratio of the noradrenaline storing cell islets of the SHR group to that of NR group in each stage

Fig. 3. NSCI in the adrenal medulla of NR at 18th day after birth (Coupland et al.'s method). The intensity of NSCI increases compared to Fig. 1. $\times 40$

Fig. 4. NSCI in the adrenal medulla of SHR at 18th day after birth (Coupland et al.'s method). The area of NSCI is about 2 times as large as that of NR. $\times 40$

birth, while the area of noradrenaline storing cell islets of control rats was 7.6 per cent on the 8th day after birth; 9.1 per cent on the 10th day after birth; 10.5 per cent on the 18th day after birth (Fig. 3); 12.7 per cent on the 30th day after birth; 11.2 per cent on the 40th to 50th day after birth; 12.1 per cent in the 5th month after birth and 14.2 per cent over one year after birth and the values of the controls were found to be almost constant. The ratios of the area of noradrenaline storing cell islets of spontaneously hypertensive rats against that of control rats were: 1.3 times on the 8th day after birth; 1.5 times on the 10th day after birth; 1.8 times on the 15th day after birth; 2.1 times on the 18th day after birth; 2.0 times on the 40th to 50th day after birth; 2.1 times

in 5-6 months after birth and 1.9 times over one year after birth. The area of noradrenaline storing cell islets of spontaneously hypertensive rats was larger on the 6th day after birth than that of control rats and as days passed, the ratio increased gradually up to about 2 times on the 18th day after birth and this state continued after the 18th day. More than one year after birth, there was a tendency of a slight decrease in these values. The increase in the area thus obtained did not always show a clear parallelism with the increase in blood pressure.

The intensity of staining of noradrenaline storing cell islets of spontaneously hypertensive rats and of control rats was weakest in both on the 6th day after birth and increased gradually

TABLE II DIMENSIONAL CHANGES OF THE NORADRENALINE STORING CELL ISLETS IN THE ADRENAL MEDULLA OF SPONTANEOUSLY HYPERTENSIVE RATS

Days or Months after Birth	No. of Animals	Sex	Mean Systolic Blood Pressure (mm. Hg)		Duration of Hypertension (Weeks)	Mean Body Weight at Sacrifice (gm) M (range)	Adrenal Weight (gm) $\times 10^5$ / Body Weight (gm)		Dimension of NSCI / Dimension of Adrenal Medulla $\times 100$		
			M	S. E.			M	S. E.	M	S. E.	#
40-50 Days	SHR	5	♂	134 $\pm$ 2.7	0	125 (110-140)	24.8 $\pm$ 1.7		22.6 $\pm$ 1.2***		$\times 2.0$
	NR	5	♂	114 $\pm$ 3.5	0	124 (120-130)	25.4 $\pm$ 0.9		11.2 $\pm$ 0.9		
5 Months	SHR	5	♂	182 $\pm$ 2.6	9-12	284 (260-310)	14.0 $\pm$ 0.6		25.6 $\pm$ 1.6***		$\times 2.1$
	NR	5	♂	123 $\pm$ 3.4	0	288 (260-310)	14.7 $\pm$ 0.5		12.1 $\pm$ 0.8		
over 12 Months	SHR	5	♂	199 $\pm$ 2.9	45-52	344 (320-370)	12.6 $\pm$ 1.1		26.6 $\pm$ 0.7***		$\times 1.9$
	NR	5	♂	118 $\pm$ 2.3	0	366 (350-380)	11.6 $\pm$ 0.7		14.2 $\pm$ 1.1		

Abbreviations are same as Table I

TABLE III DIMENSIONAL CHANGES OF THE NORADRENALINE STORING CELL ISLETS

		No. of Animals	Sex	Mean Systolic Blood Pressure (mm. Hg) M S. E.	Duration of Hypertension (Days)	Months after Birth	Mean Body Weight at Sacrifice (gm) M (range)
Prehypertensive Stage	Hypertension Renal	5	♂	132 ± 3.8	0	1.5	126 (115-135)
	Control	5	♂	113 ± 3.3	0	1.5	123 (110-130)
Early Stage	Renal Hypertension	5	♂	156 ± 2.5	1	2	141 (130-160)
	Control	5	♂	119 ± 3.7	0	2	138 (130-150)
Middle Stage	Renal Hypertension	5	♂	171 ± 3.8	75-84	5	284 (240-320)
	Control	5	♂	114 ± 3.8	0	5	284 (260-350)
Late Stage	Renal Hypertension	5	♂	169 ± 5.6	160-172	3	856 (340-380)
	Control	5	♂	115 ± 3.8	0	8	386 (360-420)

Abbreviations are same as Table I

* $0.1 < p < 0.2$

#: The dimensional ratio of the noradrenaline storing cell islets of the renal hypertension group to that of control group

until the 15th day, but thereafter, the intensity remained constant.

(2) Renal Hypertensive Rat Group

The blood pressure, body weight, adrenal weight and the area of noradrenaline storing cell islets in the adrenal medulla are shown in Table III.

Blood Pressure: While renal hypertensive rats showed hypertension (above 150 mmHg) from the 5th day after operation, control rats remained 100 to 124 mmHg. There was a significant difference between the two.

Body Weight and Adrenal Weight: There was no significant difference between renal hypertensive rats and control rats.

Histochemical and Histometrical Findings on the Noradrenaline Storing Cell Islets in the Adrenal Medulla: There was no significant change between renal hypertensive rats and control rats in the staining intensity of the noradrenaline storing cell islets in the adrenal medulla.

The area of noradrenaline storing cell islets in renal hypertensive rats was 15.5 per cent in the prehypertensive stage, 17.8 per cent in the early stage, 17.9 per cent in the middle stage and 19.1 per cent in the late stage (Fig. 6). Although an increase was noted, there was no significant difference among the areas of early

stage, middle stage and late stage. In the case

Fig. 5. NSCI in the adrenal medulla of control rat at 6th month after sham operation (Coupland et al.'s method). $\times 40$

IN THE ADRENAL MEDULLA OF RENAL HYPERTENSIVE RATS

$\frac{\text{Adrenal Weight (gm)}}{\text{Body Weight (gm)}} \times 10^5$		$\frac{\text{Dimension of NSCI}}{\text{Dimension of Adrenal Medulla}} \times 100$			Days after Operation	Mean Systolic Blood Pressure before Operation (mm. Hg)	
M	S. E.	M	S. E.	#		M	S. E.
31.3 ± 1.7		15.5 ± 0.4			4	114 ± 2.5	
28.3 ± 1.3		13.8 ± 0.8		× 1.1	4	114 ± 4.2	
30.3 ± 1.4		17.8 ± 1.1**			5-6	119 ± 4.4	
23.3 ± 0.9		13.8 ± 0.9		× 1.3	6	116 ± 2.7	
18.3 ± 1.1		17.9 ± 1.0***			90	114 ± 3.5	
15.0 ± 0.7		12.1 ± 0.5		× 1.5	90	109 ± 2.2	
14.8 ± 0.4		19.1 ± 0.9***			180	112 ± 3.8	
11.1 ± 0.5		12.3 ± 1.1		× 1.6	180	120 ± 2.3	

of control rats, the area was 13.8 per cent be-

Fig. 6. NSCI in the adrenal medulla of renal hypertensive rat at 6th month after operation (Coup-land et al.'s method). The area of NSCI is about 1.6 times as large as that of control rat. × 40

fore the development of hypertension, 13.8 per cent in the early stage, 12.1 per cent in the middle stage and 12.3 per cent in late stage (Fig. 5), and the values were found to be almost constant. The ratio of the area in renal hypertensive rats against that in control rats was 1.1 times in the prehypertensive stage, 1.3 times in the early stage, 1.5 times in the middle stage and 1.6 times in the late stage. In the case of renal hypertensive rats, there was little increase in the area of noradrenaline storing cell islets before the development of hypertension. After the development of hypertension, the area increased and after the middle stage, the area became up to about 1.5 times as large as that of control rats. However, this increase in the area did not always show a parallelism with the grade of hypertension.

(3) DCA Hypertensive Rat Group

The blood pressure, body weight, adrenal weight and the area of noradrenaline storing cell islets in the adrenal medulla are shown in Table IV.

Blood Pressure: In the case of DCA hypertensive rats, the blood pressure gradually became higher as days passed after operation: on the 21st day after operation, it was higher than 150mmHg and over 200mmHg in the 6th month after operation, while, in the case of control rats, the height was between 108 and 125mmHg. There was a significant difference between the two.

TABLE IV DIMENSIONAL CHANGES OF THE NORADRENALINE STORING CELL ISLETS

		No. of Animals	Sex	Mean Systolic Blood Pressure (mm. Hg)		Duration of Hypertension (Days)	Months after Birth	Mean Body Weight at Sacrifice (gm) M (range)
				M	S. E.			
Prehypertensive Stage	DCA Hypertension	5	♂	139 ± 4.1		0	1.5	129 (120-140)
	Control	5	♂	110 ± 2.7		0	1.5	132 (125-140)
Early Stage	DCA Hypertension	5	♂	158 ± 3.5		2-3	2	219 (150-260)
	Control	5	♂	120 ± 3.5		0	2	254 (240-280)
Middle Stage	DCA Hypertension	5	♂	187 ± 3.7		60-71	5	360 (280-390)
	Control	5	♂	114 ± 1.6		0	5	274 (240-300)
Late Stage	DCA Hypertension	5	♂	211 ± 11.1		155-159	8	348 (340-360)
	Control	5	♂	117 ± 1.9		0	8	320 (290-350)

Abbreviations are same as Table I

#: The dimensional ratio of the noradrenaline storing cell islets of the DCA hypertension group to that of control group

Body Weight and Adrenal Weight: There was no significant difference between DCA hypertensive rats and control rats.

Histochemical and Histometrical Findings on Noradrenaline Storing Cell Islets in the Adrenal Medulla: There was no significant difference between DCA hypertensive rats and control rats in the intensity of staining of noradrenaline storing cell islets in the adrenal medulla. The area of noradrenaline storing cell islets in the adrenal medulla was 16.9 per cent in the prehypertensive stage, 19.9 per cent in the early stage, 20.5 per cent in the middle stage and 20.4 per cent in the late stage (Fig. 7). Although the area increased after the development of hypertension, there was no significant difference among the values in the early stage, middle stage and the late stage. In the case of control rats, it was 14.4 per cent in the prehypertensive stage, 14.1 per cent in the early stage, 12.9 per cent in the middle stage and 12.4 per cent in the late stage. The ratios of the area in DCA hypertensive rats against that in control rats were 1.2 times in the prehypertensive stage, 1.4 times in the early stage, 1.6 times in the middle stage and 1.7 times in the late stage. However, the increase in the area did not always show a clear parallelism with the grade of hypertension.

(4) Neurogenic Hypertensive Rat Group

The blood pressure, body weight, adrenal weight and the area of noradrenaline storing cell islets in the adrenal medulla are shown in Table V.

Blood Pressure: The blood pressure of neurogenic hypertensive rats became higher than 150 mmHg on the 8th day after the 2nd operation and finally up to 200 mmHg, but thereafter as days passed, it gradually became low, 150 to 175 mmHg in the 6th month after the operation, but there was a significant difference between the neurogenic hypertensive rats and the controls.

Body Weight and Adrenal Weight: There was no significant difference between neurogenic hypertensive rats and control rats.

Histochemical and Histometrical Findings on Noradrenaline Storing Cell Islets in the Adrenal Medulla: There was no significant difference between neurogenic hypertensive rats and control rats in the intensity of staining of noradrenaline storing cell islets in the adrenal medulla. The area of noradrenaline storing cell islets of the adrenal medulla in neurogenic hypertensive rats was 15.7 per cent in the prehypertensive stage, 21.4 per cent in the early stage, 25.5 per cent in the middle stage and 25.3 per cent in the late stage (Fig. 8). Although an increase was noted, there was no significant difference between the area of middle

IN THE ADRENAL MEDULLA OF DCA HYPERTENSIVE RATS

$\frac{\text{Adrenal Weight (gm)}}{\text{Body Weight (gm)}} \times 10^5$		$\frac{\text{Dimension of NSCI}}{\text{Dimension of Adrenal Medulla}} \times 100$		Days after Operation	Mean Systolic Blood Pressure before Operation (mm. Hg)	
M	S. E.	M	S. E.		M	S. E.
30.6 ± 1.5		16.9 ± 1.0*		17	116 ± 3.1	
30.1 ± 0.9		14.4 ± 0.7		17	111 ± 2.4	
18.5 ± 0.8		19.9 ± 1.8**		23-24	120 ± 5.0	
18.0 ± 1.5		14.1 ± 0.7		23	118 ± 3.0	
12.9 ± 0.9		20.5 ± 0.8***		90	113 ± 4.3	
15.6 ± 1.1		12.9 ± 0.4		90	113 ± 2.2	
10.9 ± 0.4		20.4 ± 0.4***		180	118 ± 2.2	
13.6 ± 0.8		12.4 ± 0.4		180	118 ± 1.9	

stage and of the late stage. On the other hand, in the case of control rats, the area was 14.2 per

cent in the prehypertensive stage, 15.3 per cent in the early stage, 15.0 per cent in the middle stage and 13.7 per cent in the late stage and the

Fig. 7. NSCI in the adrenal medulla of DCA hypertensive rat at 6th month after operation (Coupland et al.'s method). The area of NSCI is about 1.7 times as large as that of control rat. $\times 40$

Fig. 8. NSCI in the adrenal medulla of neurogenic hypertensive rat at 6th month after operation (Coupland et al.'s method). The area of NSCI is about 1.8 times as large as that of control rat. $\times 40$

TABLE V DIMENSIONAL CHANGES OF THE NORADRENALINE STORING CELL ISLETS

		No. of Animals	Sex	Mean Systolic Blood Pressure (mm. Hg)		Duration of Hypertension (Days)	Months after Birth	Mean Body Weight at Sacrifice (gm) M (range)
				M	S. E.			
Prehypertensive Stage	Neurogenic Hypertension	5	♂	137 ± 2.4		0	1.5	129 (120-140)
	Control	5	♂	114 ± 3.0		0	1.5	129 (125-135)
Early Stage	Neurogenic Hypertension	5	♂	183 ± 11.4		2	2	160 (100-215)
	Control	5	♂	120 ± 4.7		0	2	157 (130-165)
Middle Stage	Neurogenic Hypertension	5	♂	163 ± 4.7		70-72	5	262 (230-320)
	Control	5	♂	121 ± 3.0		0	5	254 (200-290)
Late Stage	Neurogenic Hypertension	5	♂	165 ± 3.4		160-164	8	326 (310-350)
	Control	5	♂	109 ± 1.7		0	8	328 (300-350)

Abbreviations are same as Table I

#: The dimensional ratio of the noradrenaline storing cell islets of the neurogenic hypertension group to that of control group

values were shown to be almost constant. The ratio of the area of neurogenic hypertensive rats against that of control rats was 1.1 times in the prehypertensive stage, 1.4 times in the early stage, 1.7 times in the middle stage and 1.8 times in the late stage. That is, the area of noradrenaline storing cell islets in neurogenic hypertensive rats showed a gradual increase but the increase in the area did not show any clear parallelism with the grade of hypertension.

DISCUSSION

The study on the relation between hypertension and catecholamine began when catecholamine was extracted as peressor substrate and since GOLDENBERG¹⁹ reported that essential hypertension was caused by the increase in the amount of the secretion of noradrenaline, many medical scholars have measured the amount of noradrenaline in the urine and the blood of patients with essential hypertension, but there have been different reports on the result of such measurement. The patient with essential hypertension have been reported to excrete elevated²⁰⁻²⁵, normal²⁶⁻³³ and reduced³⁴ amount of noradrenaline in their urine. However, the general view at present is that the amount is within normal range. But recently, MAEKAWA et al.³⁵ reported cases in which neurogenic factor

was the cause of hypertension and the amount of noradrenaline excreted in the urine paralleled with the fluctuation of blood pressure.

At the present time, it is said that noradrenaline in blood and urine is produced by nerve endings of sympathetic nervous system and by adrenal medulla, but, while nerve endings of sympathetic nervous system produces only noradrenaline, adrenal medulla produces adrenaline and noradrenaline and they are stored in a separate cell, respectively. And it is reported that the COUPLAND et al.'s glutaraldehyde osmium tetroxide method stains the noradrenaline in the adrenal medulla selectively, and that the distribution of noradrenaline storing cell islets is not different from that observed by using iodate method and fluorescent method.

The area of noradrenaline storing cell islets in the adrenal medulla of spontaneously hypertensive rats is larger than that of control rats on the 6th day after birth when the staining of noradrenaline storing cell islets becomes possible, showing about 1.2 times as large as that of control rats and after that as days pass, it becomes larger and larger until the 18th day after birth when it becomes about 2 times as large, and this state remains almost same after that. That is, spontaneously hypertensive rats have about 2 times as large noradrenaline storing cell islets in the adrenal medulla before the develop-

IN THE ADRENAL MEDULLA OF NEUROGENIC HYPERTENSIVE RATS

$\frac{\text{Adrenal Weight (gm)}}{\text{Body Weight (gm)}} \times 10^5$ M S. E.	$\frac{\text{Dimension of NSCI}}{\text{Dimension of Adrenal Medulla}} \times 100$ M S. E. #			Days after Operation	$\frac{\text{Mean Systolic Blood Pressure before Operation (mm. Hg)}}{\text{M S. E.}}$	
31.6 ± 2.0	15.7 ± 0.6*	× 1.1		5	114 ± 2.3	
29.1 ± 1.0	14.2 ± 0.7			5	116 ± 3.0	
26.3 ± 2.9	21.4 ± 2.5**	× 1.4		10	118 ± 3.5	
26.3 ± 2.7	15.3 ± 0.7			10	121 ± 2.7	
14.7 ± 0.8	25.5 ± 2.1***	× 1.7		90	110 ± 2.9	
15.1 ± 0.6	15.0 ± 1.4			90	118 ± 1.7	
15.1 ± 0.4	25.3 ± 3.1***	× 1.8		180	108 ± 0.4	
14.8 ± 0.7	13.7 ± 0.2			180	113 ± 3.3	

ment of hypertension as those of control rats and this state remains after the development of hypertension. Considering the fact that the area of noradrenaline storing cell islets of the adrenal medulla in control rats keeps a constant value throughout all stages, the area of noradrenaline storing cell islets in the adrenal medulla of spontaneously hypertensive rats increases gradually after the 6th day after birth and keeps an almost constant value on and after the 18th day after birth.

This finding corresponds with the finding of TABEI¹⁰ on the area of noradrenaline storing cell islets in the adrenal medulla of spontaneously hypertensive rats, which reported that through the stage before the development of hypertension and after the development of hypertension, the area of noradrenaline storing cell islets of spontaneously hypertensive rats was about 2 times as large as that of control rats.

It is stated by OZAKI³⁶ that the content of noradrenaline in the adrenal medulla of spontaneously hypertensive rats becomes larger as blood pressure rises. SUZUKI³⁷ reported that spontaneously hypertensive rats have a larger amount, 1.8 to 2 times, of noradrenaline in the adrenal medulla than that of control rats in the prehypertensive stage and also early hypertensive stage. These findings almost correspond with the histometrical findings mentioned above.

As the cause of increase in the area of nor-

adrenaline storing cell islets in the adrenal medulla, two factors can be considered. One is the increase of synthesis of noradrenaline, and the other is the reduction of the release and the dissolution of noradrenaline.

TABEI¹⁰ showed histochemically the increased activity of monoamine oxidase of heart muscle of spontaneously hypertensive rats. On the other hand, OZAKI³⁶ reported that the heart muscle of spontaneously hypertensive rats contained a large amount of noradrenaline than that of control rats. This seems to show an increase in metabolism of noradrenaline and in its dissolution. However, OZAKI³⁸ reported that the biochemical estimation of the activity of catechol-O-methyl transferase in various organs proved no difference between spontaneously hypertensive rats and control rats. Therefore, on this point, that is, on the cause of the increase in the area of noradrenaline storing cell islets, further study is required. This problem will be studied in the next report.

It is said that there is no difference among the content of noradrenaline in the adrenal medulla of renal hypertensive dogs, that of neurogenic hypertensive dogs and that of normotensive dogs³⁹. DE SCHAEPE-DRYVER et al.⁴⁰ also reported that there was no difference between the content of noradrenaline in the adrenal medulla of renal hypertensive rats and that of control rats. However, some essential hypertensive patients were reported to have a

greater quantity of noradrenaline in the heart than normotensive persons⁴¹. OSHIMA et al.⁴² observed the increase of noradrenaline excretion in urine of renal hypertensive dogs. MANGER et al.⁴³ reported the increase of epinephrine-like substance in the blood of hypertensive dogs induced by occlusion of carotid arteries.

Now, in this experiment, it was proved that the area of noradrenaline storing cell islets in the adrenal medulla of renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats did not show significant increase before the development of hypertension but that it tended to increase remarkably after the development of hypertension. That is, the ratio of the area of noradrenaline storing cell islets in renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats against that of control rats was 1.1 times, 1.2 times and 1.1 times, respectively, before the development of hypertension. Although there was no remarkable increase before the development of hypertension, it increased after the development of hypertension by 1.3 times, 1.4 times and 1.4 times, respectively and up to 1.6 times, 1.7 times and 1.8 times 6 months after the development of hypertension.

Thus, in the case of renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats, the area of noradrenaline storing cell islets in the adrenal medulla increases after the development of hypertension and the condition is quite different from the case of spontaneously hypertensive rats that have a larger area, 2 times, of noradrenaline storing cell islets before the development of hypertension than the normotensive controls.

When the area values of renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats in all stages are compared, it is found that the largest is the middle and late stage in the cases of neurogenic hypertensive rats. These values in neurogenic hypertension had no difference from the area values of noradrenaline storing cell islets in spontaneously hypertensive rats that have about 2 times as large an area as that of control rats. In contrast to this, in the case of renal hypertensive rats and DCA hypertensive rats, even in the late stage the area value does not exceed that of

noradrenaline storing cell islets in spontaneously hypertensive rats: i.e., the area values of the noradrenaline storing cell islets of the adrenal medulla in spontaneously hypertensive rats are closest to that of neurogenic hypertensive rats. This suggests that the neurogenic factor may be concerned with the development and the maintenance of hypertension of spontaneously hypertensive rats and in this field, there are some reports by OKAMOTO et al.^{44,45}. As the cause of the increase in the area of noradrenaline storing cell islets of the adrenal medulla in renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats, the increase in noradrenaline synthesis and the reduction of the secretion and the dissolution of noradrenaline can be considered.

Noradrenaline is synthesized from tyrosine by the action of 4 enzymes: tyrosine hydroxylase, aromatic 1-amino acid decarboxylase, dopamine-beta-oxidase and phenylethanolamine-N-methyl transferase, and it is said that the control of the speed of noradrenaline synthesis, tyrosine hydroxylase and dopamine-beta-oxidase function as rate limiting steps, and also that the availability of the substrate, the availability of enzyme cofactors and the structural integrity of the subcellular particles influence the speed control. It is also said that this tyrosine hydroxylation is facilitated by the impulse of sympathetic nerve system⁴⁶, and on the other hand, it is reported that the secretion of noradrenaline increases when splanchnic nerve is stimulated and decreases when this nerve is cut⁴⁷⁻⁴⁹. From these facts, it is supposed that for the synthesis of noradrenaline and the secretion of noradrenaline, the sympathetic nervous system plays an important role. McCUBBIN reported that the function of sympathetic nervous system increased in renal hypertensive dogs⁵⁰. It can be supposed that in the case of renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats, both the synthesis and the secretion of noradrenaline increase, but on this point further study in detail is required.

SUMMARY

Spontaneously hypertensive rats, renal hypertensive rats, DCA hypertensive rats, neuro-

genic hypertensive rats and control rats totaling 360 were killed under minimum stress. The noradrenaline in the adrenal medulla was stained by COUPLAND et al.'s glutaraldehyde osmium tetroxide method and the dimensional changes of noradrenaline storing cell islets were studied. In this experiment, spontaneously hypertensive rats were divided into four stages through their survival duration: until 30th day after birth, the 40th to 60th day after birth, the 5th to 7th month after birth and over one year after birth. Renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats were divided into four stages: the prehypertensive stage, the early stage of the 5th to the 24th day after operation, the middle stage of the 3rd month after operation and late stage of the 6th month after operation. Findings in spontaneously hypertensive rats, renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats were compared with those in control rats with almost the same survival duration.

(1) The noradrenaline storing cell islets in the adrenal medulla became stainable from the 6th day after birth in both cases of spontaneously hypertensive rats and control rats. After that, the intensity of staining became stronger and after the 15th day, it maintained constant intensity.

(2) The area of noradrenaline storing cell islets of the adrenal medulla in spontaneously hypertensive rats on the 6th day after birth was 9.6 per cent, about 1.2 times as large as that of control rats (8.1%) and after that as days passed, the area became larger. On the 10th day, that of spontaneously hypertensive rats was 14.1 per cent, about 1.5 times as large as that of control rats (9.1%), on the 18th day, 21.7 per cent, about 2 times as large as that of control rats (10.5%), and after that the state was constant until one year after birth, when it decreased a little. Spontaneously hypertensive rats develop hypertension in the 8th week after birth while the area of noradrenaline storing cell islets in the adrenal medulla becomes about 2 times as large as that of control rats before the development of hypertension, and this state remains constant after the development of hypertension.

(3) As for the area of noradrenaline storing cell islets in the adrenal medulla of renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats, the following results were obtained:

(a) In the prehypertensive stage, the areas in three kinds of hypertensive rats did not increase as in the case of spontaneously hypertensive rats, and there was no significant difference from that of control rats.

(b) However, the area of noradrenaline storing cell islets in the adrenal medulla increased immediately after the development of hypertension. (i) Renal hypertensive rats showed an increase in the area; 17.8 per cent in the early stage, 17.9 per cent in the middle stage and 19.1 per cent in the late stage, and their values were 1.3 times, 1.5 times and 1.6 times as large as that of control rats. (ii) In the case of DCA hypertensive rats, the area was 19.9 per cent in the early stage, 20.5 per cent in the middle stage and 20.4 per cent in the late stage, showing an increase of 1.4 times, 1.6 times and 1.7 times in comparison with that of control rats. (iii) In the case of neurogenic hypertensive rats, the area was 21.4 per cent in the early stage, 23.5 per cent in the middle stage, 26.3 per cent in the late stage, and was 1.4 times, 1.7 times and 1.8 times as large as that of control rats, respectively, that is, in renal and DCA hypertensive rats, the grade of increase in the area was always smaller than that of spontaneously hypertensive rats but neurogenic hypertensive rats showed almost the same value as that of spontaneously hypertensive rats in the late stage.

(c) The intensity of staining of noradrenaline storing cell islets did not show any difference from those of control rats and did not change remarkably in parallel with the length of hypertension.

(4) The increase in the area of noradrenaline storing cell islets in the adrenal medulla of spontaneously hypertensive rats, renal hypertensive rats, DCA hypertensive rats and neurogenic hypertensive rats did not show clear parallelism with the grade of hypertension.

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