Studies on the Thyroid in Spontaneously Hypertensive Rats

I. Thyroid Activity*

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OKAMOTO and AOKI have successfully separated from Wistar rats a colony of hypertensive rats in which all of the animals develop severe hypertension spontaneously without any treatment. These are named "Spontaneously Hypertensive Rats"^{1,2} (hereafter abbreviated as SHR). This seems to be the animal counterpart of human essential hypertension because of its spontaneous occurrence^{1,2} and lack of a renal mechanism^{1,3-6}. Considerable evidence has been accumulated which can be interpreted as indicating the participation of the autonomic nervous system⁷⁻¹⁵ and the endocrine system^{7,15-20} in the development and maintenance of the hypertension.

With respect to the endocrine organs, in addition to the pituitary¹⁶ and the adrenal cortex^{7,16} the participation of the thyroid gland has been suggested. AOKI et al.¹⁶ reported increases in the thyroid weight, follicular cell height, follicular cell area and follicular lumen area in SHR which corresponded to "struma parenchymatosa macrofollicularis". Later, TABEI⁷ demonstrated elevated acid phosphatase activity histochemically in the follicular cells of SHR. Furthermore, AOKI found that hypophysectomy or thyroidectomy prevented the development or maintenance of spontaneous hypertension¹⁷ which was restored by the administration of thyroid hormone.¹⁹ These facts probably indicate that thyroid activity is elevated in SHR, although more precise functional study seems to be required, and that the thyroid gland plays some role in the development and maintenance of hypertension in SHR.

In the present study, the elevated thyroid ac-

tivity of SHR was firmly established with the use of radioactive iodine. Thyroid activity was also determined in this study in rats with renal infarction hypertension or desoxycorticosterone acetate (DCA) hypertension. At the same time the effect of propylthiouracil (PTU), an antithyroid drug, on the blood pressure of SHR and control rats was also studied, and on the basis of these results the relationship between thyroid activity and blood pressure is discussed.

MATERIALS

A total of 127 (85 male and 42 female) SHR of various ages were used in this study. They consisted of one male F₁₀, four male F₁₁, 25 male and three female F₁₂, 30 male and 25 female F₁₃, 17 male and 11 female F₁₄, five male and three female F₁₅-SHR and three male SHR obtained by mating male F₁₅ with female F₁₄-SHR. Among them, the numbers of ten animals were 10168♂, 13102♂, 130083♀, 130094♀, 130106♀, 130107♀, 130108♀, 130114♀, 130223♀, and 130225♀. One hundred and six SHR were the offspring of 10009♂, 10029♂, 10060♂, 10087♂, 10169♂, 110049♂, 110055♂, 110112♂ and/or 110113♂, and 10017♀, 10058♀, 10069♀, 10080♀, 10172♀, 10178♀, 10225♀, 110063♀, 110071♀, 110077♀, 110089♀, 110114♀, 110115♀, 110117♀, 110119♀ and/or 120090♀, eight F₁₅-SHR were the offspring of 140236♂, and 140180♀ or 140224♀, and the last three were the offspring of 150071♂ and 140180♀. These numbers described here are registered in the pedigree in this laboratory. The pedigree up to F₁₂ has been diagramed in previous papers^{2,21}

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TABLE I THYROIDAL ¹³¹I UPTAKE IN SHR

Age	Sex		No. of Animals	Blood Pressure (mmHg)	Body Weight (g)	3-Hour Uptake (%)	24-Hour Uptake (%)	Thyroid Weight / Body Weight (mg/g)
13-16 Days	Male	SHR	7		31 ± 3.1†	2.3 ± 0.44†ns		0.130 ± 0.0211†ns
		Controls	6		29 ± 5.4	2.3 ± 0.52		0.128 ± 0.0167
	Female	SHR	5		26 ± 4.9	2.4 ± 0.24†ns		0.132 ± 0.0197ns
		Controls	6		24 ± 4.2	2.6 ± 1.06		0.137 ± 0.0114
40-60 Days	Male	SHR	10	139 ± 4.1†***	164 ± 26.6	8.9 ± 2.06***		0.086 ± 0.0177ns
		Controls	10	116 ± 5.4	168 ± 27.8	4.8 ± 2.09		0.081 ± 0.0149
	Female	SHR	9	134 ± 6.0***	123 ± 23.1	8.3 ± 2.43**		0.088 ± 0.0121ns
		Controls	9	114 ± 2.1	122 ± 25.2	5.4 ± 1.56		0.099 ± 0.0137
4-6 Months	Male	SHR	5	171 ± 16.8***	293 ± 21.9	18.3 ± 3.89***		0.073 ± 0.0066***
		Controls	4	172 ± 16.8***	298 ± 27.2		36.5 ± 4.94***†	
	Female	SHR	7	127 ± 7.4	303 ± 26.2	9.1 ± 1.56		0.057 ± 0.0089
		Controls	7	126 ± 7.7	305 ± 13.2		22.4 ± 4.42	
12-14 Months	Male	SHR	4	162 ± 10.6***	217 ± 13.4	14.5 ± 3.52*		0.112 ± 0.0057ns
		Controls	5	122 ± 6.0	209 ± 17.9	8.4 ± 2.62		0.105 ± 0.0107
	Female	SHR	6	172 ± 9.8***	357 ± 39.4	7.9 ± 1.79**		0.065 ± 0.0095ns
		Controls	8	128 ± 4.2	425 ± 51.2	4.7 ± 0.93		0.058 ± 0.0083
18-20 Months	Male	SHR	6	192 ± 28.4***	246 ± 23.3	10.6 ± 1.54*		0.085 ± 0.0148ns
		Controls	9	117 ± 7.5	239 ± 23.1	8.4 ± 2.22		0.080 ± 0.0114
	Female	SHR	3	178 ± 18.3**	357 ± 21.7	6.1 ± 1.68**		0.100 ± 0.0256ns
		Controls	3	119 ± 3.6	380 ± 34.6	2.3 ± 0.34		0.058 ± 0.0118

†: Mean ± standard deviation

ns, *, ** and ***: Differences from the control values are statistically not significant, significant at the levels of 0.05 > P > 0.01, 0.01 > P > 0.001 and 0.001 > P, respectively.

TABLE II RATE OF ^{131}I RELEASE FROM THE THYROID IN SHR

Age	Sex		No. of Animals	Blood Pressure (mmHg)	Body Weight (g)	^{131}I Release Rate (%/day)
40–60 Days	Male	SHR	8	$137 \pm 4.2^\dagger$	$100 \pm 6.8^\dagger$	$10.5 \pm 0.51^{\dagger***}$
		Controls	9	114 ± 3.6	104 ± 7.2	8.7 ± 1.47
	Female	SHR	7	130 ± 3.4	139 ± 11.6	$9.4 \pm 0.79^{***}$
		Controls	6	110 ± 4.9	139 ± 11.5	5.6 ± 0.98
4–6 Months	Male	SHR	9	177 ± 8.1	300 ± 14.4	$9.9 \pm 1.29^*$
		Controls	13	126 ± 7.2	322 ± 19.5	8.0 ± 1.84
	Female	SHR	6	166 ± 10.0	208 ± 12.7	$10.4 \pm 1.37^{***}$
		Controls	6	120 ± 4.2	216 ± 13.5	6.2 ± 1.00
12–14 Months	Male	SHR	6	179 ± 18.1	373 ± 34.5	9.1 ± 0.98^{ns}
		Controls	8	133 ± 4.0	410 ± 28.0	9.1 ± 0.68
	Female	SHR	5	176 ± 17.4	252 ± 34.4	6.4 ± 1.05^{ns}
		Controls	8	120 ± 3.4	252 ± 22.3	6.3 ± 1.30

† : Mean $\pm$ standard deviation

ns, *, **, and ***: Differences from the control values are statistically not significant, significant at the levels of $0.05 > P > 0.01$, $0.01 > P > 0.001$ and $0.001 > P$, respectively.

TABLE III CONVERSION RATIO IN ADULT MALE SHR

Age	Sex		No. of Animals	Blood Pressure (mmHg)	Body Weight (g)	24-Hour Conversion Ratio (%)
7 Months	Male	SHR	5	$191 \pm 17.1^\dagger$	$340 \pm 17.6^\dagger$	$74.0 \pm 5.04^{\dagger*}$
		Controls	8	132 ± 7.4	337 ± 22.2	64.5 ± 6.27

† : Mean $\pm$ standard deviation

*: Difference from the control is statistically significant at the level of $0.05 > P > 0.01$.

TABLE IV THYROID HORMONE SECRETION RATE IN MALE SHR

Age	Sex		No. of Animals	Blood Pressure (mmHg)	Body Weight (g)	Prediction Equation	Thyroid Hormone Secretion Rate (μg L-thyroxine/100 g body weight/day)
40–60 Days	Male	SHR	13	$136 \pm 6.6^\dagger$	$195 \pm 25.3^\dagger$	$Y = 8.84X + 72.81$	3.08
		Controls	13	114 ± 6.4	189 ± 20.9	$Y = 6.27X + 85.95$	2.24
4–6 Months	Male	SHR	8	176 ± 10.6	350 ± 22.8	$Y = 8.69X + 74.89$	2.89
		Controls	10	125 ± 4.4	371 ± 37.7	$Y = 7.78X + 80.92$	2.45
12–14 Months	Male	SHR	9	178 ± 14.2	381 ± 33.1	$Y = 9.16X + 74.89$	2.74
		Controls	11	126 ± 5.6	421 ± 36.5	$Y = 10.42X + 77.58$	2.15

† : Mean $\pm$ standard deviation

A total of 155 (106 male and 49 female) normotensive Wistar rats of the same ages were used as controls. These rats were supplied from the Wistar strain maintained in the Animal Center of Kyoto University, from which SHR were separated.²

Among these rats, 116 SHR and 142 control rats were used for 135 and 162 measurements of thyroid activity (Tables I, II, III and IV), respectively; i.e., some of them were used twice. These animals were divided into three groups according to age, namely, 40 to 60 days, 4 to 7 months and

12 to 14 months of age, which in SHR corresponds to prehypertensive (below 150 mmHg), early hypertensive and advanced hypertensive stages, respectively. In addition, groups of rats 13 to 16 days and 18 to 20 months of age were added in the uptake test.

Eleven SHR and 13 control rats 4 to 10 months of age were used for the propylthiouracil experiment.

To produce renal infarction and DCA hypertension, a total of 51 normotensive male Wistar rats weighing 110 to 180 g at the time of operation were used. These rats were also obtained from the Animal Center of Kyoto University. As controls, 61 normotensive Wistar rats from the same source were used.

All these rats were kept in a temperature- and humidity-controlled room with artificial lighting. By means of a photo tube, lighting time was set approximately from sunrise to sunset. The temperature was set at 24°C and the humidity at 50%. They were fed *ad lib.* on Oriental stock chow diet "NMF" (Oriental Yeast Co.) except for the animals in which the thyroid hormone secretion rate was tested which were fed on CLEA's stock chow diet "CA-1" (Japan CLEA Co.). They were given tap water to drink except for the DCA implanted rats which were given 1% sodium chloride solution during the development of hypertension and the PTU-treated rats which were given PTU solution.

METHODS

Thyroid activity was measured by the 3- or 24-hour ^{131}I uptake by the thyroid, the rate of ^{131}I release from the gland, the 24-hour conversion ratio and the thyroid hormone secretion rate.

(1) *^{131}I uptake by the thyroid:* Animals were injected intraperitoneally with 0.05 μCi of carrier-free Na^{131}I . Three or 24 hours later they were killed by bleeding from the aorta under light ether anaesthesia. Thyroid glands were removed and weighed, and the radioactivity was measured in a well-type scintillation counter (Shimadzu ES 7-EC 10A). It was adjusted for isotopic decay, and the uptake was expressed as the percentage of the dose administered.

(2) *Rate of ^{131}I release from the thyroid:* This was determined by WOLFF's method²² Animals received a single intraperitoneal injection of 5 μCi of carrier-free Na^{131}I . Radioactivities over the neck and epigastric regions were counted under light ether anaesthesia by means of exter-

nally placed collimated scintillation counter with animals placed on their backs and all legs tied to a board. The opening of the lead shield was 2.2 cm in diameter, and the distance between these regions and the scintillation crystal was 5 cm. In order to avoid geometric errors of the neck position, the average of three to six counts was taken by replacement of the animal's neck. The initial neck count was made 60 hours after the ^{131}I injection, and four more counts at 48 hour intervals were made thereafter. Each count was corrected by one-half the epigastric count,²² by the general background and also for isotopic decay. Serial counts were plotted semilogarithmically, the slope of each curve was calculated by the method of least squares and the rate of ^{131}I release was expressed as the percentage of the release in a day (%/day).

(3) *Conversion ratio:* The 24-hour conversion ratio was determined by the method of VAN MIDDLESWORTH et al²³ with some modification. Animals received ca. 10 μCi of carrier-free Na^{131}I intraperitoneally 24 hours before sacrifice. Blood was obtained from the abdominal aorta under ether anaesthesia. Two milliliter of plasma was used for the determination. The total plasma ^{131}I was counted in the well-type scintillation counter. Then, two drops of 3% NaI carrier and 5 ml of 20% trichloroacetic acid were added and centrifuged. The precipitate was washed twice with 5 ml of 10% trichloroacetic acid. The radioactivity of the precipitate was counted. The conversion ratio was calculated as follows:

$$\text{Conversion ratio} = \frac{\text{Count/min of precipitate}}{\text{Count/min of total plasma } ^{131}\text{I}} \times 100$$

(4) *Thyroid hormone secretion rate:* The thyroid hormone secretion rate was estimated by the method of REINEKE and SINGH²⁴ except for the administration of propylthiouracil. Animals received 5 μCi of carrier-free Na^{131}I , and the neck counts were taken by the above-mentioned technique. L-Thyroxine (Sigma) in 0.0004N NaOH solution was injected subcutaneously twice daily. The dose was increased progressively at two day intervals. Doses of 0, 0.5, 1.0 and 1.5 μg L-thyroxine/100 g body weight/day were used. Starting 60 hours after the ^{131}I injection, neck counts were taken just before each increase in thyroxine dosage at 48 hour intervals. Each count was expressed as a percentage of the preceding count, plotted against thyroxine dosage and the prediction equation of the curve was calculated by the

method of least squares. When the percentage (Y) was taken as 100, the estimated thyroid hormone secretion (X) was obtained (Table IV).

Renal infarction hypertension, as described by LOOMIS²⁵ was induced by bilateral ligation of the posterior branches of the main renal arteries. These animals were not given sodium chloride in their drinking water. In this study hypertension developed in about two thirds of the operated animals. Since it was found that among the animals which showed normotension for three weeks, hypertension never developed, operated normotensive animals of this kind were carefully prepared and served as "sham operation" controls (Table V).

*DCA hypertension*²⁶ was induced by the implantation of a 25 mg DCA pellet (Merck) in the back muscle of unilaterally nephrectomized rats. They were given 1% sodium chloride solution to drink. To prepare animals in the advanced stage of DCA hypertension, however, sodium chloride solution was withdrawn after about 1.5 months, since these animals showed very severe hypertension, frequently over 200 mmHg, and could not survive long enough and since thyroid activity would be influenced by large amounts of sodium chloride ingestion itself.²⁷ The hypertension was not affected by the withdrawal.²⁸ On the other hand, in the early DCA hypertension group sodium chloride was given, because it is necessary for the development of hypertension.²⁹ Control animals were prepared by the same procedure but without pellet implantation.

In the *propylthiouracil (PTU) experiment*, a 2.5×10^{-4} solution of 6-propyl-2-thiouracil (Tokyo Kasei) was prepared by dissolving it in hot water, and this was administered as drinking water. Animals drank 20 to 25 ml of the solution per day which contained about 5 to 6 mg of PTU.

Systolic blood pressures were measured by the tail-water plethysmographic method without anesthesia.²

Pituitary and thyroid glands were weighed on a torsion balance.

Statistical analyses of the data were performed using the "t" test for the 95% confidence limit.

RESULTS

1. Thyroid activity in SHR

(1) ¹³¹I uptake by the thyroid gland (Table I)

Animals were divided into five groups according to age, i.e., 13 to 16 days, 40 to 60 days, 4 to 6 months, 12 to 14 months and 18 to 20 months of age. The blood pressure some days before the

measurement, body weight at sacrifice, ¹³¹I uptake and thyroid weight/body weight ratio are shown in Table I. As shown in the table, the ¹³¹I uptake was significantly higher in SHR than in control rats in both sexes and in all age groups except for the group aged 13 to 16 days, in which there was also no difference in the thyroid weight/body weight ratio.

The blood pressure of the 13- to 16-day-old rats could not be measured because they were too small for the apparatus. As shown in Table I, although the 40- to 60-day-old SHR did not show hypertension (above 150 mmHg), there was already a significant difference in the blood pressure between SHR and control rats. SHR over 4 to 6 months of age were hypertensive while control rats were not. Among these animals, all of the 18- to 20-month-old SHR showed mild to moderate lesions of periarteritis nodosa in the mesenteric vessels.

(2) Rate of ¹³¹I release from the thyroid (Table II)

The rate of ¹³¹I release was determined in 40- to 60-day-old, 4- to 6-month-old, and 12- to 14-month-old rats of both sexes. Table II shows the increase in the rate of ¹³¹I release in 40- to 60-day-old and 4- to 6-month-old SHR. In 12- to 14-month-old rats, however, there was no significant difference by this method.

(3) Conversion ratio (Table III)

The 24-hour conversion ratio was determined in 7-month-old male rats only. As shown in Table III, it was significantly higher in SHR than in control rats.

(4) Thyroid hormone secretion rate (Table IV)

It was determined using 8 to 13 male rats in each group. Prediction equations and thyroid hormone secretion rates are shown in Table IV. Thyroid hormone secretion rates were always larger in SHR than in control rats in these age groups. On the basis of these results, the percentage increases in SHR compared with control rats are 37%, 18% and 27% for 40- to 60-day-old, 4- to 6-month-old and 12- to 14-month-old rats, respectively.

2. Thyroid activity in renal infarction and DCA hypertension

In this experiment animals were divided into two groups according to the stage of hypertension, namely, the early and the advanced stages. In the early stage, the duration of hypertension was less than two weeks, and the blood pressure was still increasing. The advanced stage corres-

TABLE V THYROIDAL ^{131}I UPTAKE, PITUITARY AND THYROID WEIGHT AT THE ADVANCED STAGES OF RENAL INFARCTION HYPERTENSION

Stage		No. of Animals	Blood Pressure (mmHg)	Body Weight (g)	3-Hour Uptake (%)	24-Hour Uptake (%)
Early Stage	Renal Infarction	10	$176 \pm 18.8^\dagger$	$198 \pm 20.0^\dagger$	$6.6 \pm 1.12^{\dagger ns}$	
	Hypertension	3	164 ± 16.5	208 ± 25.6		$19.2 \pm 2.02^\dagger$
	Sham Operation	10 2	129 ± 8.2 133	194 ± 19.0 209	6.9 ± 1.58	18.5
Advanced Stage	Renal Infarction	9	177 ± 15.8	356 ± 40.4	8.1 ± 1.88^{ns}	
	Hypertension	4	174 ± 17.8	364 ± 44.7		13.7 ± 1.33^{ns}
	Sham Operation	16 7	129 ± 5.4 130 ± 10.0	376 ± 31.3 378 ± 46.7	8.6 ± 1.74	11.6 ± 3.65

$\dagger$: Mean $\pm$ standard deviation

*: Difference from the control is statistically significant at the level of $0.05 > P > 0.01$.

ns: Not significant

α : No. of animals examined

TABLE VI THYROIDAL ^{131}I UPTAKE, PITUITARY AND THYROID WEIGHT AND THE ADVANCED STAGES OF DCA HYPERTENSION IN THE

Stage		No. of Animals	Blood Pressure (mmHg)	Body Weight (g)	3-Hour Uptake (%)	24-Hour Uptake (%)
Early Stage	DCA	9	$175 \pm 15.9^\dagger$	$176 \pm 26.0^\dagger$	$6.6 \pm 1.34^{\dagger ns}$	
	Hypertension	4	180 ± 9.4	191 ± 36.7		$16.4 \pm 6.78^{\dagger ns}$
	Sham Operation	10 6	120 ± 5.4 119 ± 4.5	189 ± 18.2 211 ± 10.3	7.3 ± 2.26	16.5 ± 1.79
Advanced Stage	DCA	9	197 ± 7.8	337 ± 32.2	8.2 ± 2.09^{ns}	
	Hypertension	3	206 ± 9.2	372 ± 47.2		13.2 ± 3.22
	Sham Operation	8 2	125 ± 6.4 129	354 ± 28.5 344	8.4 ± 0.68	12.0

$\dagger$: Mean $\pm$ standard deviation

*: Difference from the control is statistically significant at the level of $0.05 > P > 0.01$.

ns: Not significant

α : No. of animals examined

THE EARLY AND
IN THE RAT

<i>Pituitary Weight</i> <i>Body Weight</i> (mg/g)	<i>Thyroid Weight</i> <i>Body Weight</i> (mg/g)
$0.033 \pm 0.0218^{+ns}$ (10) $^{\alpha}$	$0.091 \pm 0.0125^{+ns}$ (10) $^{\alpha}$
0.031 ± 0.0035 (7)	0.090 ± 0.0126 (10)
0.025 ± 0.0237^{ns} (11)	$0.064 \pm 0.0066^{**}$ (13)
0.025 ± 0.0016 (13)	0.058 ± 0.0076 (21)

AT THE EARLY
RAT

<i>Pituitary Weight</i> <i>Body Weight</i> (mg/g)	<i>Thyroid Weight</i> <i>Body Weight</i> (mg/g)
$0.030 \pm 0.0021^{+ns}$ (7) $^{\alpha}$	$0.090 \pm 0.0166^{+ns}$ (12) $^{\alpha}$
0.031 ± 0.0037 (7)	0.088 ± 0.0151 (16)
0.026 ± 0.0033^{ns} (12)	$0.072 \pm 0.0115^{*}$ (12)
0.026 ± 0.0022 (9)	0.056 ± 0.0211 (10)

ponded with the stage 2.5 to 3 months after the development of hypertension in which hypertension had already been longstanding; in the case of DCA hypertension, it was selfsustaining by this time.

(1) Thyroid activity in renal infarction hypertension (Table V)

As seen in Table V, the ^{131}I uptake test showed no significant difference between renal hypertensive rats and control rats in either the early or the advanced stages. The fact that there was no difference in 3-hour and also in 24-hour uptakes seems to indicate the possibility that there was no difference also in the rate of ^{131}I release from the thyroid. The thyroid weight/body weight ratio, however, increased slightly and significantly in the advanced stage group. There was no difference in the pituitary weight. Periarthritis nodosa could not be found in the mesenteric vessels of these animals.

(2) Thyroid activity in DCA hypertension (Table VI)

The results were almost the same as in the above experiment with renal hypertensive rats, that is, the ^{131}I uptake and the pituitary weight/body weight ratio showed no significant difference, while the thyroid weight/body weight ratio increased significantly in the advanced stage. Ten out of 12 DCA hypertensive rats in the advanced stage showed mild to moderate periarthritis nodosa in the mesenteric vessels, but these lesions were not so severe as those frequently seen in DCA hypertensive rats given sodium chloride solution continuously until sacrifice. The general condition was also better than in the latter, and there was no decrease in body weight, as seen in the table. Periarthritis nodosa was not found in the sham operation control rats.

3. Effect of propylthiouracil on the blood pressure of SHR and control rats (Fig. 1)

Four- to 10-month-old rats were used in this experiment. The change of blood pressure caused by propylthiouracil (PTU) administration is illustrated in Fig. 1. Changes in body weight (average values) of these animals were as follows. In SHR, 370 g, 359 g, 359 g, 349 g and 330 g, and in control rats, 405 g, 390 g, 393 g, 383 g and 359 g, at the start and at the end of the first, second, fourth and eighth weeks, respectively. As some of these animals were sacrificed for another purpose in the course of the experiment, measurement at the end of the eighth week could be performed in only five SHR and six control rats. The average

Fig. 1. Effect of prolonged Propylthiouracil administration on blood pressure in SHR control rats.

values of the initial blood pressure and body weight in these rats were 185 mmHg and 343 g in five SHR, and 123 mmHg and 365 g in six control rats.

As illustrated in Fig. 1, PTU-treated SHR and control rats both showed a gradual decrease in blood pressure during the experiment, and the differences between them were always significant statistically, although the differences became smaller with time; that is, they were 59, 48, 52, 44 and 36 mmHg at the start and at one, two, four and eight weeks of PTU treatment, respectively.

DISCUSSION

Thyroid activity was assessed in four ways in this study: i.e. ^{131}I uptake by the thyroid gland, rate of ^{131}I release from the thyroid gland, the 24-hour conversion ratio and the thyroid hormone secretion rate. In the ^{131}I uptake test, 3-hour uptake was used chiefly not only because it represents pure uptake with minimal ^{131}I release from the gland but also because it may be less dependent on diminished renal function, which was expected in some experimental cases in this study, than 24-hour uptake. In order to minimize

the error in vitro counts were used. As seen in the data of old SHR (Tables I and II) the uptake test seemed to be more sensitive in determining thyroid activity than the rate of ^{131}I release. This was another reason why 3-hour uptake was used also in the renal infarction and DCA hypertension experiment. The rate of ^{131}I release is widely accepted as a better method of measuring thyroid activity than ^{131}I uptake. It is directly related to thyroid hormone secretion, and less affected by changes in renal function.^{30,31} The 24-hour conversion ratio, which is one of the sensitive methods for diagnosing human hyperthyroidism, is concerned with the turnover and release of thyroidal iodine and is increased in cases of hyperthyroidism. The thyroid hormone secretion rate was estimated according to the principle of PERRY³² and REINEKE and SINGH²⁴ which represents the minimal exogenous thyroxine required to inhibit thyroidal ^{131}I release completely. This inhibition can be explained by the effect of thyroxine on pituitary thyrotropic hormone secretion. Although it is expressed as "L-thyroxine/100 g body weight/day", it corresponds not to the real "thyroxine" secretion rate but to the "thyroid hormone" secretion rate calculated in

terms of L-thyroxine. In this study REINEKE and SINGH's method²⁴ for small groups of animals was used rather than PERRY's³² which requires a great number of animals. The thyroid hormone secretion rates in control rats obtained in this study are in agreement with REINEKE and SINGH's estimate²⁴ of 2.15 μg L-thyroxine/100 g body weight/day for adult male albino rats, but somewhat deviated from the values of ca. 5 μg obtained by PERRY³² of 5.2 μg obtained by DEMPSEY and ASTWOOD³³ using a goiter prevention technique, of ca. 1.5 μg by TAUROG and CHAIKOFF³⁴ using a method involving specific activities of diiodotyrosine and thyroxine in the thyroid, and of 0.97 μg by GREGERMAN³⁵ using the radioactive turnover technique in which only thyroxine was measured. These differences seem to depend on the method used. As a rough estimate, the thyroid hormone secretion rate decreased with age in control rats as well as in SHR in this study, which corresponds to the observations by GRAD and HOFFMAN³⁶ and NARANG and TURNER³⁷ in normal female rats.

From the data obtained in the functional study of the thyroid of SHR at various ages (Tables I, II, III and IV), elevated thyroid activity was firmly established in 40- to 60-day-old and 4- to 6-, 12- to 14- and 18- to 20-month-old SHR, the ages of which corresponded to pre-, early, advanced and far advanced hypertensive stages, respectively. These results are in agreement with the previous histometric and histochemical studies on the thyroid of SHR by AOKI et al.¹⁶ and by TABEL⁷. In SHR the values of thyroid hormone secretion rate are about 1.2 to 1.4 times greater than in the controls. This may well indicate the degree of increased thyroid activity in SHR. Elevated thyroid activity in SHR, however, was not found as judged from the 3-hour ¹³¹I uptake and the thyroid weight/body weight ratio at 13 to 16 days of age, at which time thyroid function is said to reach a second peak after birth and the adult type integration of the pituitary gland with hypothalamus seems to occur.³⁸ These results indicate that recognizable thyroid activation occurs between 16 and 40 days of age in SHR. The maximum difference of the thyroid hormone secretion rate between SHR and controls was found at 40 to 60 days of age, when the rate of increase of blood pressure is greatest in SHR.^{2,21} These facts suggest that the thyroid gland plays a role in the development of this spontaneous hypertension.

AOKI et al.¹⁶ found some increases in the pitui-

tary weight and in the percentage of basophils in the anterior lobe starting in the prehypertensive stage in SHR. Moreover, histochemical studies of the hypothalamus showed hyperfunction of some nuclei which seemed to have a TSH activating function.²⁰ These facts suggest that the increased thyroid activity in SHR is due to increased activity in the hypothalamus-pituitary-thyroid system.

Thyroid activity in the early (within two weeks after the development of hypertension) and advanced (over 2.5 months' duration of hypertension) stages of renal infarction and DCA hypertension was estimated by 3- and 24-hour ¹³¹I uptakes. The reason that 3-hour uptake was chiefly used has already been explained. In this experiment, the thyroid weight/body weight ratio was also calculated. There were some discrepancies between the weight and the uptake (Tables V and VI). In such cases the data of the uptake should be regarded as important. It is known that the ingestion of large amounts of sodium chloride increases thyroid activity.²⁷ Since one of the purposes of this experiment was to observe the effect of high blood pressure itself on thyroid function, rats with renal infarction were fed no sodium chloride, and in the advanced stage of DCA hypertension, sodium chloride in drinking water was withdrawn about one month before the determination of thyroid activity, when the hypertension had become self-sustained.²⁸ But, in early DCA hypertension sodium chloride is necessary for the development of hypertension,²⁹ so it was given to the DCA-implanted rats, and as controls nephrectomized rats given sodium chloride solution were prepared. In contrast to SHR in the previous experiment, ¹³¹I uptake was not elevated in either the early or the advanced stage of these two kinds of hypertension. The increase in the 3-hour uptake in advanced stages of both sham operation and DCA or renal infarction hypertension seems to be physiological, since normal rats showed the same tendency with age, as seen in Table I. These results indicate first that the hypertensive state probably does not cause increased thyroid activity. Second, elevated thyroid activity seems not to be necessary to develop and maintain either DCA or renal infarction hypertension. FREGLY³⁹ showed increased thyroid activity as assessed by thyroid weight, thyroid acinar cell height and conversion ratio in rats made hypertensive by enclosing both kidneys in latex, and concluded that the thyroid played an important secondary role in the development of hypertension over 160 mmHg. The present ex-

periment, however, did not confirm this, although experimental animals showed high blood pressure over 160 mmHg when the thyroid activity was determined in the early stage.

As reported by many investigators, thyroidectomy or the administration of the antithyroid drug, propylthiouracil (PTU), prevents the development and maintenance of many kinds of experimental hypertension especially in the rat: i.e., DCA,⁴⁰ renal,⁴¹⁻⁴³ adrenal regeneration,^{44,45} and salt⁴⁶ hypertension. AOKI reported that radiothyroidectomy as well as methylthiouracil administration prevented the development and maintenance of hypertension in SHR,¹⁷ and later he also reported that radiothyroidectomy lowered the blood pressure slightly (about 15 mmHg lower than in controls at the end of the eighth week) in normotensive young female rats.¹⁸ FREGLY et al.⁴⁷ reported thyroidectomy or PTU also reduced the systolic blood pressure of normal rats. These facts indicate that the thyroid gland plays some role in the maintenance of blood pressure in experimental hypertensive rats as well as in normal rats. In the present experiment on the effect of prolonged administration of small doses of propylthiouracil on the blood pressure of SHR and control rats, it was also observed. However, it was also found that the level of blood pressure in SHR was still significantly higher than in control rats even at the end of the eighth week. This fact indicates that the thyroid does not seem to be the only factor in the maintenance of high blood pressure in SHR, and that there must be other factor(s) involved in the difference of blood pressure between SHR and normal rats.

SUMMARY

1. Elevated thyroid activity was observed in 40- to 60-day-old and 4- to 7-, 12- to 14- and 18- to 20-month-old SHR in at least one of the following four tests using radioactive iodine: the thyroidal ¹³¹I uptake, the rate of thyroidal ¹³¹I release, the conversion ratio and the thyroid hormone secretion rate. The thyroid hormone secretion rate was increased 37%, 18% and 27%, respectively in 40- to 60-day-old, and 4- to 6- and 12- to 14-month-old SHR. In 13- to 16-day-old SHR, however, neither the thyroidal ¹³¹I uptake nor the thyroid weight/body weight ratio differed from those in the control rats.

2. In the early (acute) and the advanced (chronic) stages of renal infarction and DCA hypertension, thyroid activity as assessed by the thyroidal ¹³¹I uptake did not differ from that in

the sham-operated control rats, although increased thyroid weight/body weight ratios were observed in advanced stages.

3. Prolonged propylthiouracil (PTU) administration effectively reduced the blood pressure of both adult male SHR and control rats. The blood pressure of SHR was always higher than that of control rats during PTU administration.

4. On the basis of these results, the relationship between hypertension and thyroid activity, especially in SHR, is discussed.

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