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Excessive Thyrotropin Concentrations in the Circulation of the Spontaneously Hypertensive Rat[1] (38679)

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The hypertension of spontaneously hypertensive Wistar rats (SHR) has proven to be an especially appealing model for investigators since the disorder is genetically transmitted to 100% of the offspring. Moreover, cardiovascular lesions quite similar to those occurring in human benign and malignant essential hypertension are ultimately produced (1). The genesis of the hypertension, however, remains undetermined.

Okamoto (1) reported results which he felt indicated a derangement of pressor and depressor mechanisms of the hypothalamus which caused hyperactivity of the medulla oblongata and sympathetic nervous system in SHR. Furthermore, he suggested that there was mild hyperfunction of the hypophyseo-adrenocortical and hypophyseo-thyroidal systems, due to disturbed hypothalamic control over secretion of adrenocorticotrophin (ACTH) and of thyrotropin (TSH). This hyperactivity of the neuroendocrine systems was postulated to produce and maintain hypertension in SHR (1).

The present work was designed to repeat our initial studies of thyroid function in SHR, but using Kyoto-bred, normotensive rats as controls, instead of the American-bred normotensive Wistar rats which served as controls in the early work (2, 3). An excess of circulating thyrotropin was again consistently observed in SHR over controls, though serum thyroxine (T4) and triiodothyronine (T3) concentrations relative to the two types of controls, revealed differences.

Materials and Methods. Groups of inbred SHR and inbred normotensive Wistar rats from Kyoto (K) (Expt. B) and random-bred normotensive Wistar rats from the United States (N) (Expt. A) were maintained on Purina rat chow, containing 0.49% sodium and 1.73 parts of iodine per million of diet, and tap water *ad libitum*. The rats were housed under the same conditions. SHR and N were originally obtained from Purina, later from Carworth Laboratories. K were originally obtained from the National Institutes of Health (courtesy of Dr. Carl Hansen).

Systolic blood pressures were measured in unanesthetized rats by a tail cuff technique employing a pulse transducer (E and M Instrument Co., Houston, TX) and recorded on a Grass Polygraph. Heart rates were obtained by counting systolic pulsations recorded on the polygraph. Blood was obtained for determination of T3, T4 and TSH from the inferior vena cava of animals anesthetized with sodium pentobarbital administered intraperitoneally (50 mg/kg).

Rat thyrotropin was determined by radioimmunoassay using rat TSH and antirat TSH serum obtained from the National Pituitary Agency and prepared by Dr. A. F. Parlow. The method was essentially that given by Dr. Parlow with the assay kit, except for the radioiodination step. In our hands, greater reproducibility was obtained by iodinating with 1 mCi per 5 μg rat TSH instead of the 1–2 mCi per 2.5 μg rat TSH suggested by Parlow. Most samples were assayed in triplicate.

Rat serum triiodothyronine concentrations initially were determined in duplicate by the radioimmunoassay method of Rubenstein, Butler and Werner, employing ethanol extraction to denature thyroxine binding globulin (TBG) (4). Recoveries of labelled T3 were somewhat less than those from human

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serum, averaging 72.5% as opposed to 93%, correction for losses being made accordingly. Later, a modification of the original procedure which provided greater rapidity of assay (5) was employed. Recoveries with ethanol extraction in the rapid method approached 100%.

Serum thyroxine was initially determined in duplicate by the chemical method of Benotti (6). Later, determinations were made by the rapid radioimmunoassay procedure used for both T3 and T4 determinations (5).

Results. The original experiment (Expt. A) was conducted with eight male SHR and seven male Wistar control rats from this country (N) (Fig. 1). TSH concentrations, expressed as μunit/ml, were significantly higher (*t* test, *P* 0.005) in SHR, 167 ± 36.6 μunit/ml, than in N, 27 ± 2.6 μunit/ml. T4 concentrations were significantly lower (*P* 0.001) in SHR, 2.15 ± 0.46 μg/dl, than in N, 4.0 ± 0.92 μg/dl, whereas T3 concentrations were similar in both groups. 50.9 ± 2.3 and 48.4 ± 3.3 ng/dl, respectively.

Experiment B was similar to Experiment A, but used 15 male SHR and 14 Kyoto male controls (K), together with three female SHR and three female Kyoto controls (K) (Fig. 1). A marked elevation of circulating TSH concentrations similar to that in Experiment A was observed in the SHR compared to K. For SHR males, TSH concentrations, expressed as ng/ml, averaged 891.1 ± 133.4 whereas that for K males was 186.2 ± 28.3 ng/ml (*P* 0.001). Serum T4 concentrations were greater in SHR males than in K males, 3.0 ± 0.1 μg/dl and 2.3 ± 0.1 μg/dl, respectively (*P* 0.001). Serum T3 concentrations possibly also were greater, 92.4 ± 3.0 ng/dl versus 82.4 ± 1.9 ng/dl, respectively (*P* 0.025). In the females, however, although the TSH excess was again found, T4 concentrations were virtually the same in SHR and K, whereas T3 concentrations were higher in K than SHR.

Three SHR and three K bloods were tested for thyroxine binding protein concentrations, electrophoretically. No difference was observed between the two groups. In addition to albumin binding of radioactive T4, a small postalbumin peak was observed in both groups.

Morphological findings in thyroid glands of various groups of Wistar rats. Nineteen N thyroids were compared with the glands of 20 SHR of varying ages. The glands were examined grossly but were not weighed. They appeared comparable in size. However, Fregley has reported thyroid wt/body wt ratios higher for SHR than for N (7). The following parameters were evaluated: follicular size, nature of colloid, height of follicular lining cells, and presence of inflammatory cell infiltrate. The mean thyroid follicular size in SHR was greater, height of lining cells shorter, and alveolar colloid often more inspissated and/or fragmented, with the presence of macrophages (colloidophages), in contrast to the thyroids of N, which displayed smaller follicles, more normal colloid and somewhat taller lining cells than those of SHR.

The thyroid glands of 8 K and 5 SHR were similarly analyzed. The glands were examined grossly and were considered to be of comparable size. Aoki (8) has reported thyroid wt/body wt ratios to be higher for SHR than for K. Again, follicular size in SHR tended to be somewhat greater than in K, but lining cell heights were comparable. The colloid appeared more granular in SHR and more homogeneous in K. One thyroid from an SHR showed peripheral vacuolization of the colloid.

Discussion. The choice of controls for SHR studies has varied. Some investigators have used normotensive American Wistar rats (N) bred brother to sister as controls for SHR studies (9). However, the use of random-bred normotensive Wistar rats as controls minimizes the possibility of introducing some consistent biochemical deviation resulting from inbreeding and unrelated to hypertension. Breeding SHR with N in order to obtain less hypertensive or normotensive rats that can be used for comparative studies has also been reported (10). Recent evidence suggests that offspring of the normotensive Wistar strain (K) used by Okamoto resemble most closely SHR (11, 12) and hence appear to be the best controls for studies of SHR.

In our initial experiment, American-bred healthy Wistar rats (N) were employed as controls. It was recognized that a study with

	T_3 ng/dl	T_4 μg/dl	TSH μU/ml* or ng/ml ϒ
SHR 5.5 mos. B.P 163±4			
(n) ♂	(8)	(8)	(7)
MEAN	50.88	2.15	167.37 *
SEM	±2.29	±.46	±36.56
N 5.5 mos. B.P. 128±4			
(n) ♂	(7)	(7)	(6)
MEAN	48.43	4.04	26.98*
SEM	±3.33	±.92	±2.63
P (SHR vs N)	NS	<.001	<.005
SHR 5-8 mos. B.P. 163±4			
(n) ♂	(15)	(15)	(15)
MEAN	92.41	3.01	891.13 ϒ
SEM	±2.99	±0.13	±133.41
K 5-7.5 mos. B.P. 116±2			
(n) ♂	(14)	(14)	(14)
MEAN	82.43	2.34	186.21 ϒ
SEM	±1.94	±0.05	±28.27
P (SHR vs K)	<.025	<.001	<.001
SHR 5.5 mos. B.P. 153±3			
(n) ♀	(3)	(3)	(3)
MEAN	65.33	2.17	675.0 ϒ
RANGE	58-77	1.94-2.46	370-1175
K 5.5 mos. B.P. 120±8			
(n) ♀	(3)	(3)	(3)
MEAN	101.33	2.28	88.33 ϒ
RANGE	92-107	1.77-2.61	50-130

FIG. 1. Table showing mean concentrations ± SEM for T3, T4 and TSH concentrations in the Experiments A (comparison of SHR with N), and B (comparison of SHR with K), of the text. Although *P* values are presented, the number of rats per experiment is small. Values for TSH marked with an asterisk are expressed as μunit/ml whereas values marked with the Y-like symbol are expressed as ng/ml.

Kyoto control rats (K) was necessary, and this was undertaken as soon as K rats became available. Okamoto and associates had reported the thyroid gland of SHR to be hyperfunctioning (1), secondary to hypothalamic stimulation of the pituitary from excessive release of TSH (so-called "tertiary hyperthyroidism").

In our recent studies, evidence for tertiary hyperthyroidism is contradictory. The excess

of TSH in SHR compared to N noted previously was clearly confirmed when K were used for controls. Yet, only slight elevations of T4 and T3 serum concentrations were noted in male SHR compared to K; and in three female SHR, these values were actually decreased relative to their respective K controls. These results in turn differ from the decreased T4 and normal T3 concentrations suggestive of hypothyroidism, found when SHR were compared to N.

As stated, Okamoto and coworkers interpreted the histologic and electron microscopic changes they found in the thyroid as indicative of thyroidal hyperfunction. Their rats were housed at 18–25°. In their original experiments, the rats were fed a diet of wheat, dried sardine and vegetables (1), though other diets were also used in later work. The iodine content of the diets was not specified. Our rats were maintained at 22°, and were fed Purina Rat Chow. Since the latter contains 1.73 parts of iodine per million, a 30–40 g daily food intake would provide about 50–70 μg iodine per day, a high iodine intake. If the Kyoto diet were significantly lower in iodine content than the present Purina diet, an effect of iodine in suppressing hyperfunction of the thyroid in our experiments would have to be considered. This would decrease follicular cell height and level of function of a hyperfunctioning thyroid.

After an initial blockade of the incorporation of iodine by the thyroid cells, a maintained excess of iodine would permit overcoming the blockade, with renewed turnover of iodine and hormone production, the Wolff-Chaikoff effect (13). Hyperfunction of the thyroid would not be consistent with the excess of TSH we observed in the SHR, unless a hypothalamic derangement with oversecretion of TRH were able to overcome the well-known inhibition by thyroid hormone excess of the anterior pituitary response to TRH.

The histologic evidence from our studies fits with the concept of an iodine-suppressed or relatively inactive thyroid in SHR. The sections do not reveal the classical morphological alterations of a hyperactive thyroid gland, such as increased height and number of follicular lining cells with papillary infoldings, and diminished size of follicles with thin colloid and peripheral vacuolization. Rather, the lining cells were not enlarged, follicular size was increased and colloid was inspissated.

Aoki and coworkers (8) observed enlargement of thyroid follicles in SHR, but in addition described an increase in the number and size of the lining cells. This increase in cells was not confirmed in any of the 25 animals studied in our laboratory. Aoki observed the decreased volume of stored colloid in SHR, also noted in our series. We interpreted the latter as due to increased viscosity of colloid and partial removal by macrophages.

On morphological grounds, therefore, it appears that the thyroid glands of SHR are not hyperfunctioning, but could possibly be hypoactive. However, the differences between thyroid glands of SHR and K are slight and may not be significant. An elevated TSH concentration in this circumstance might represent a compensatory response of the anterior pituitary designed to maintain homeostasis.

Another explanation besides tertiary hyperthyroidism, and primary hypothyroidism, as discussed, for an increase in TSH concentrations with minimally increased T3 and T4 values, could be that the radioimmunoassay for TSH is detecting α and β subunits of TSH as well as TSH. It might also be that a TSH secreting tumor was present within the anterior pituitary. No such neoplasm was evident to gross inspection of our SHR at autopsy. Moreover, Okamoto and coworkers' careful study of the anterior pituitary revealed no tumors, although they claimed evidence for possible pituitary hyperfunction (1).

A final explanation for TSH excess might be the occurrence of peripheral resistance to the thyroid hormones (14, 15). In this disorder, described in man but not as yet in the experimental animal, TSH concentrations are not especially high, whereas there is an abnormal elevation of thyroid hormone concentrations, without hyperthyroidism.

A conclusive explanation for the present findings appears to be wanting. Further work is suggested.

Summary. Thyroid function in SHR was compared to that of inbred normotensive Wistar rats from Kyoto, and random bred Wistar rats from the United States. A marked increase in circulating thyrotropin concentration was found in SHR compared to controls. Serum thyroxine and T3 concentrations relative to controls, however, were variable and the concentrations in male and female SHR relative to Kyoto controls did not correspond with each other. No ready explanation for the circulating excess of thyrotropin, with minimal increase in T4 and T3 concentrations, is available.

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