Disturbance of Thyroidal Iodine Metabolism in BB/W Rat

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Abstract. To investigate the thyroid function in Bio-Breeding Worcester (BB/W) rats, we have
examined the iodine metabolism, serum TSH and thyroid hormone levels in 8- and 16-week-old BB/W
and normal Wistar (W) rats. At 8 weeks of age, serum TSH levels were significantly higher in BB/W rats
than in W rats, although there was no difference in the serum levels of free T3 and free T4.
Furthermore, the thyroidal radioactive iodine incorporation at 48 h was significantly lower in BB/W rats,
suggesting that they might have some defects in iodine organification. At 16 weeks of age, serum TSH
levels were also significantly higher in BB/W rats than in W rats. Furthermore, serum TSH levels in
16-week-old BB/W rats were significantly higher than in 8-week-old BB/W rats. The thyroid weight was
significantly greater in BB/W rats, probably due to the increased serum TSH. The thyroidal radioactive
iodine uptake at 48 h and the iodine content in the thyroid homogenates were significantly lower in
BB/W rats. These results suggest that BB/W rats have some defect in iodine metabolism resulting in
impaired thyroid hormone synthesis.

Key words: BB/W rat, Lymphocytic thyroiditis, Iodine organification, Thyroid function.

(Endocrinol Japn 38: 647-653, 1991)

BIO-BREEDING Worcester (BB/W) rats spontaneously develop insulin-dependent diabetes mel-
litus [1-5] and about 60% of them also develop lymphocytic thyroiditis (LT) [6-9]. We have pre-
viously shown that such are an animal model of Hashimoto’s thyroiditis because of the histological
resemblance [8, 9].

It has been reported that patients with Hashi-
moto’s thyroiditis frequently develop hypothyroid-
ism during the ingestion of large quantities of
iodine [10], and that they have a defect in the
intrathyroidal organification of iodine as dem-
onstrated by a positive perchlorate discharge test [11].
With BB/W rats, Allen et al. have shown that iodide administration induced a significant increase in
the incidence of LT [12].

In this study, we measured thyroidal iodine
incorporation, serum thyroid hormone, TSH and
iodinated protein in thyroid extracts to investigate
iodine metabolism in BB/W rats. We found that 16
week-old BB/W rats had a low iodine incorpora-
tion, low thyroidal iodinated protein content, and
a high serum TSH concentration, suggesting the
presence of defective iodine organification in the thyroid.
Materials and Methods

Animals

BB/W rats were kindly donated by Dr. A. A. Like (University of Massachusetts Medical School, Worcester, USA). They were bred and maintained in our laboratory (Saitama-Tokyo colony). We have previously shown that the incidence of diabetes mellitus was 86% and that of LT was 48% at the age of 20 weeks [8]. All BB/W rats were tested for glycosuria (Tes-tape; Eli Lilly and Co., Indianapolis, Indiana) and ketonuria (Ketostix, Ames Division, Miles Laboratories, Inc., Elkhart, Indiana) between 1300 and 1500 h every day. Rats were considered to be diabetic if they had 2 (+) glycosuria. Diabetic rats were maintained on long-acting insulin (Ultralente, Novo, Vagevard, Denmark) given daily by subcutaneous injection at the following doses: 4U, 5U, or 6U for 2(+), 3(+) or 4(+) glycosuria, respectively.

Our previous studies have shown that the infiltration of lymphocytes into the thyroid develops after 10 weeks of age [8]. Therefore, we studied 8-week-old BB/W rats (n=12) which should not have histological lymphocytic infiltration, 16-week-old BB/W rats (n=9) which should have it, and two groups of age-matched normal Wistar rats (W, n=8 and n=9).

Measurement of radioactive iodine uptake (RAIU) and analysis of thyroidal iodoproteins

After being fed a low-iodine diet for one week, 111 KBq (3.0 μCi) of 131I (Dupont/NEN Research products) was injected into the peritoneal cavity. All rats were bled and thyroidectomized after 48 h. The thyroid glands were then homogenized in 1/60 M phosphate buffered saline (PBS), pH 7.4, using a glass homogenizer. Radioactivity in the homogenate was measured and the thyroidal RAIU at 48 h (48-%RAIU) was calculated.

Thyroid homogenate was then centrifuged at 10,000×g for 30 min. Almost all radioactivity was recovered in the supernatant. The supernatants were then applied to a Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (10×500 mm), which was equilibrated with 1/60 M PBS, and the radioactivity in all fractions was measured. Radioactive iodine incorporation in each fraction was expressed as the ratio of the radioactivity in that fraction to the radioactivity administered.

In the 16-week-old rats, the iodine content in the fractions with the highest radioactivity was measured with arsenious and ceric reagents [13].

Hormone assays

Serum samples from all rats were assayed for free T3 (FT3), free T4 (FT4), and TSH in duplicate by radioimmunoassay (RIA) at the same time in random order. Commercial kits were used for the FT3 and FT4 assays (Amerlex FT3 RIA and FT4 RIA kits; Amersham Japan Co., Tokyo). Serum TSH levels were measured by RIA with rat TSH and anti-rat TSH antiserum donated by the NIH.

Statistical analysis

All data are presented as the mean±SD. Statistical analysis was performed by unpaired Student's t-test.

Results

Thyroid gland size

In 8-week-old BB/W rats, there was no difference in the thyroid weight (g×10⁵)/body weight(g) ratio, as shown in Fig. 1. However, there was a decrease in actual thyroid weight in 8-week-old BB/W rats as compared with that in age-matched W rats, although this difference was not statistical-
In 16-week-old BB/W rats, the thyroid weight (g/10^5)/body weight (g) ratio was significantly increased (BB/W: 10.0±0.7 vs W: 3.6±0.4, P<0.01). The actual thyroid weight also tended to be greater in BB/W rats than in W rats (22.8±5.8 mg vs 19.3±3.4 mg, NS).

Thyroid radioactive iodine uptake

As shown in Fig. 2(A), the 48-%RAIU by the thyroid of BB/W rats was significantly decreased as compared with that for W rats at both 8 weeks (BB/W: 10.7±7.4% vs W: 30.7±12.9%, P<0.01) and 16 weeks of age (BB/W: 13.4±9.8% vs W: 26.5±9.8%, P<0.01).

When the %RAIU per 10 mg of thyroid weight was compared (Fig. 2(B)), there was a significant difference between the two groups of 16-week-old rats (6.4±1.9% vs 13.9±1.2%, P<0.01) but not between the groups of 8-week-old rats.

Iodinated proteins in thyroid homogenate

Figure 3 shows the chromatographical analysis of the supernatant of thyroid homogenate obtained from representative animals. There was no difference between the protein concentration (OD at 280 nm) in BB/W rats and that in W rats.

Nevertheless, the radioactive iodine incorporation was lower in 8-week-old BB/W rats than in W rats (Fig. 3A). In 16-week-old rats (Fig. 3B), there was a further decrease in radioactive iodine incorporation in BB/W rats as compared with the age-matched W rats (BB/W: 0.4% vs W: 1.9%). Furthermore, radioactive iodine incorporation in 16-week-old BB/W rats was considerably decreased as compared with that in 8-week-old BB/W rats (0.9% vs 0.4%).

Iodine content in the fraction with the peak radioactivity

We then measured the iodine content in the peak fraction of radioactivity in 16-week-old rats. These was a greater decrease in iodine content in BB/W rats than in W rats (Fig. 4).

Thyroid function

There was no significant difference between the serum FT3 and FT4 levels in BB/W rats and those in W rats (Fig. 5). However, there was a significant difference between the serum TSH levels in these groups. BB/W rats had significantly higher serum TSH levels than W rats regardless of their age (Fig. 6). Furthermore, serum TSH levels in 16-week-old BB/W rats were significantly higher than those in 8-week-old BB/W rats.
Fig. 3. Analysis of iodinated proteins by Sephacryl S-300 column chromatography in representative 8-week-old rats (A) and 16-week-old rats (B). There is no difference between BB/W rats and age-matched control W rats in the protein concentration shown as closed circles. Radioactive iodine incorporation (% radioactivity = CPM ÷ Total CPM) shown as open circles was lower in BB/W rats than in W rats.

Discussion

The present study has shown that the serum TSH level in BB/W rats is already significantly higher at 8 weeks of age than in age-matched W rats. In addition, the 48 h-%RAIU was also significantly decreased in 8-week-old BB/W rats. These findings also held true in 16-week-old BB/W rats. Furthermore, as shown in the chromatographical analysis, the organified radioactive iodine concentration in the thyroid from BB/W rats appeared to be lower than in the thyroid from age-matched W rats. Actually, the iodine concentration in the fraction of thyroid homogenate, that was considered to include thyroglobulin, was significantly lower in BB/W rats than in W rats. In contrast with the low 48 h-%RAIU in BB/W rats, our preliminary studies have shown that 8-week and 16-week-old BB/W rats had increased radioactive iodine uptake at 3 h when compared with the age-matched W rats. This suggests that the iodine trapping in the thyroid in BB/W rats was not defective. These observations indicate that BB/W rats have some iodine organification abnormalities even early in life.

Enlargement of the thyroid gland in 16-week-
old BB/W rats may have been due to stimulation by high serum TSH levels in addition to lymphocytic infiltration into the thyroid gland. The serum levels of thyroid hormones were normal in BB/W rats, indicating that they were subclinically hypothyroid. Allen et al. have also shown that serum TSH increased in BB/W rats after the development of thyroiditis [14]. In contrast with our study, however, they did not find a significant difference in serum TSH levels in BB/W rats before and after the onset of thyroiditis. This may have been due to the difference between their and our colonies of BB/W rats.

The abnormal thyroid hormone metabolism that is noted in BB/W rats may be at least partly due to the abnormalities of iodine metabolism
shown in this study. In contrast to our findings, Rajatanvin et al. noted no difference between BB/W rats with and without thyroiditis in the 2 h thyroidal RAIU performed during a discharge test with “phenazon”, an inhibitor of iodine organification [15]. Since the 2 h thyroidal RAIU does not accurately reflect the iodine organification even though “phenazon” was used, their results do not necessarily indicate the absence of an iodine organification-defect in BB/W rats. Furthermore, they did not compare BB/W and W rats. Since we have shown that BB/W rats may have some abnormalities in iodine organification even before the onset of overt thyroiditis, it appears to be hard to evaluate the iodine metabolism of these rats without using an appropriate control.

In contrast to BB/W rats, the thyroid glands of obese strain chickens (OS chickens, an animal model of autoimmune thyroiditis), show abnormally high iodine uptake even before the onset of thyroiditis [16]. Furthermore, this iodine uptake is not suppressed by the administration of T4, suggesting the OS chicken thyroid is extremely sensitive to a small amount of TSH or is functioning autonomously.

Although the type of thyroidal uptake abnormality differs, both BB/W rats and OS chickens appear to have some abnormalities of iodine metabolism. Previous reports and the present study taken together suggest that the thyroid itself may be abnormal in animals prone to autoimmune thyroiditis. Studies of iodine metabolism in these animals should therefore contribute to our understanding of abnormal iodine metabolism in patients with Hashimoto’s thyroiditis. Further studies are necessary to elucidate the precise mechanism of the defect in iodine metabolism in BB/W rats.

References