Pituitary-thyroid axis in short- and long-term experimental diabetes mellitus

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Abstract

Short-term experimental diabetes mellitus (DM) produces a significant decrease in serum thyroid hormones, a decreased or normal serum thyroid-stimulating hormone (TSH) and a reduction in hepatic and renal T4-5'-deiodination. However, little is known about the effects of chronic diabetes mellitus on the pituitary-thyroid axis function. We evaluated the changes induced by very short-term (6 days), short-term (15 days) and chronic (6 months) streptozotocin-induced diabetes mellitus in 3-month old female Dutch-Miranda rat serum T4, serum TSH and T4-5'-deiodinase activity in the thyroid and pituitary glands. Serum hormones were determined by specific radioimmunoassays. Iodothyronine-5'-deiodinase activities were assayed in the thyroid and pituitary microsomal fractions using 2 µM T4 as substrate. Mean serum T4 was significantly decreased from 3.3 to 2.0 µg/dl 6 days after diabetes mellitus induction, and from 2.2 to 1.5 µg/dl after 15 days of DM, with no significant changes in serum TSH, indicating a decreased pituitary TSH responsiveness to the diminished suppression by T4, even though pituitary T4-5'-deiodinase activity was unchanged. Thyroid T4-5'-deiodinase was unchanged after 6 days of diabetes mellitus, but was significantly increased from 20.6 to 37.0 pmol T3/mg protein after 15 days. Six months after diabetes mellitus induction, both serum T4 and thyroid T4-5'-deiodinase returned to normal ranges and serum TSH was unchanged, although pituitary T4-5'-deiodinase was now significantly decreased from 2.7 to 1.7 pmol T3/mg protein. These findings indicate that some kind of adaptation to chronic insulinopenia may occur at the thyroid level, but this does not seem to be true for the pituitary.

Introduction

There is a well-known relationship between thyroid diseases and diabetes mellitus (DM) in humans and animals. An impaired function of the hypothalamus-pituitary-thyroid axis, including reduced hypothalamic TRH, decreased serum TRH, TSH, T4, and T3 (1-3), low thyroid peroxidase activity (4), as well as decreased hepatic and renal T4-5'-deiodination have been reported in short-term streptozotocin-induced diabetes mellitus (3,5,6). The hypofunction of the thyroid gland in diabetic animals has generally been ascribed to decreased serum TSH (1-3), although in some studies serum TSH has been found to be normal (4,5). Pituitary T4-5'-deiodinase (DI) activity has also been re-
ported to be either normal or reduced in short-term experimental DM (3,6). However, little is known about the thyroid deiodinase activity or about the effects of chronic diabetes mellitus on thyroid function and T₄-deiodinating activities.

The present study was undertaken in order to compare the effects of short-term and chronic DM on thyroid function and regulation, with special emphasis on thyroid and pituitary T₄-5’-deiodinase activity.

### Material and Methods

Female Dutch-Miranda rats were maintained in a temperature-controlled room (22-25°C) with a light/dark cycle of 12/12 h, receiving commercial pellet chow (Purina) and water *ad libitum*. At the age of 3 months, DM was induced with a single *ip* injection of streptozotocin (Sigma, St. Louis, MO) dissolved in 0.5 ml 50 mM citrate buffer, pH 4.5. The dose of streptozotocin used was 55 mg/kg body weight in the very short- and short-term studies (6 and 15 days) and 30 mg/kg body weight in the chronic experiments (6 months).

Control (C) and diabetic (DM) rats were weighed just before the induction of DM and at the end of each experimental period. Blood glucose levels were determined 48 h after the streptozotocin injection and at the end of the experimental period using a Dextrostix/Glucometer (Ames, Elkhart, IN); random glycemia determinations were also performed during the experiment. No insulin was given to any of the diabetic animals.

Six days, 15 days and 6 months after diabetes induction, diabetic and control rats were anesthetized with ether, blood was collected from the jugular vein and glycemia was immediately determined. Thyroid and pituitary glands were rapidly removed, weighed, pooled (5-6 glands per pool), and stored at -70°C for a maximum period of 48 h. Serum was stored at -20°C for T₄ and TSH determination by specific radioimmunoassays (RIA). Serum TSH was measured using a kit supplied by NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases, Baltimore, MD) and is reported in terms of the preparation (RP-2) provided with the kit.

### 5’-Deiodinase activity assay

Previously described methods (7-10) with minor modifications were used for the determination of thyroid and pituitary DI. Briefly, the pooled thyroid glands were homogenized in 50 mM Tris-HCl buffer, pH 7.4, containing 250 mM sucrose and 5 mM DTT. The pooled pituitaries were homogenized in 50 mM Tris-HCl buffer, pH 7.6, containing 10 mM DTT. The homogenates were centrifuged at 15,000 g and 4°C for 30 min. The supernatant was centrifuged twice at 100,000 g and 4°C for 60 min, and the washed pellet was resuspended in sucrose-free homogenizing buffer. The thyroid microsome fraction was diluted to a protein concentration of 20-50 µg/100 µl, and the pituitary microsome fraction to 50-80 µg/100 µl. Protein concentrations were deter-

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**Table 1 - Effect of streptozotocin-induced diabetes on rat body weight variation, gland weights and glycemia.**

Diabetes mellitus (DM) was induced by a single *ip* injection of streptozotocin: 6- and 15-day DM rats received 55 mg, and the 6-month DM group received 30 mg of the drug. Results are reported as mean ± SEM. N, Number of experimental units of 5-6 rats each. ΔBody weight is the difference between weight at the end of the experimental period and initial body weight. *N = 1.*

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>ΔBody weight (g)</th>
<th>Thyroid (mg)</th>
<th>Pituitary (mg)</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>8</td>
<td>-18.2 ± 4.1</td>
<td>13.4 ± 1.0</td>
<td>10.8 ± 1.0</td>
<td>319 ± 22</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>4.1 ± 1.4</td>
<td>13.5 ± 1.4</td>
<td>10.6 ± 1.6</td>
<td>120 ± 5</td>
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<tr>
<td>15 days</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>14</td>
<td>-21.8 ± 9.2</td>
<td>11.5 ± 1.4</td>
<td>9.7 ± 2.0</td>
<td>328 ± 11</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>9.2 ± 2.1</td>
<td>11.6 ± 2.1</td>
<td>10.5 ± 2.6</td>
<td>112 ± 8</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>12</td>
<td>13.5 ± 3.2</td>
<td>11.9 ± 3.2</td>
<td>10.2 ± 2.3</td>
<td>290 ± 34</td>
</tr>
<tr>
<td>Control</td>
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<td>39.8 ± 0.9</td>
<td>13.6 ± 0.9</td>
<td>11.6 ± 1.3</td>
<td>132 ± 12</td>
</tr>
</tbody>
</table>
detected by the method of Bradford (11).

The assay mixture contained 100 µl microsome fraction and 2 µM T₄ in a final volume of 125 µl and was incubated at 37°C for 20 min for the thyroid assay and for 60 min for the pituitary assay. The T₃ formed was determined in the alcohol extracts by a specific RIA (12) and the results were corrected for extraction efficiency. DI activity is reported as pmol T₃ formed per mg microsome fraction protein (pmol T₃/mg protein).

Statistical analysis

Results are reported as mean ± SEM. Serum TSH values were analyzed after logarithmic transformation. Two-way analysis of variance, complemented by multiple comparison tests when appropriate (13), was used for statistical analysis of the data (SuperANOVA program, Abacus Concept, Berkeley, CA).

Results

The very short- and short-term DM animals had a significant body weight loss during the experimental period, while in the chronic DM rats the body weight gain after 6 months was about one third of that of the controls. On the other hand, the thyroid and pituitary gland weights were not significantly different in any of the DM groups. Plasma glucose levels were significantly increased in all DM groups, being 2.3-fold higher than in the controls. There was no difference in blood glucose levels between the very short-term, short-term and chronic DM rats, in spite of the different doses of streptozotocin used (Table 1).

No significant changes in serum TSH levels were observed in any of the DM groups, although there was a slight decrease after 6 and 15 days of DM (Figure 1A). Serum T₄ was significantly decreased after 6 days (C: 3.27 ± 0.34 µg/dl, DM: 2.03 ± 0.29 µg/dl, P<0.01) and 15 days (C: 2.24 ± 0.32 µg/dl, DM: 1.53 ± 0.13 µg/dl, P<0.01) of DM, but not in chronic DM (C: 2.19 ± 0.19 µg/dl, DM: 2.16 ± 0.34 µg/dl) (Figure 1B).

After 6 days of DM induction, neither pituitary DI (C: 1.38 ± 0.51; DM: 2.40 ± 0.74 pmol T₃/mg protein) nor thyroid DI (C: 13.58 ± 1.05; DM: 13.98 ± 1.71 pmol T₃/mg protein) was significantly changed (Figure 2A and 2B). Pituitary DI remained unchanged after 15 days of DM induction (C: 2.94 ± 0.47; DM: 2.57 ± 0.50 pmol T₃/mg protein), whereas thyroid DI was significantly increased (C: 20.58 ± 3.78; DM: 37.04 ± 3.73 pmol T₃/mg protein; P<0.01) (Figure 2A and 2B). After 6 months of DM, thyroid deiodinase activity was not significantly different compared to the control group (C: 32.39 ± 7.01; DM: 23.80 ± 5.54 pmol T₃/mg protein) (Figure 2B), whereas pituitary DI was decreased (C: 2.74 ± 0.90; DM: 1.71 ± 0.44 pmol T₃/mg protein, P<0.05) (Figure 2A).
Discussion

Although the causes of thyroid dysfunction in DM are still unknown, it has been shown that the metabolic alterations caused by DM, or the lack of insulin itself, can directly affect some aspects of thyroid function (14). We used different doses of streptozotocin to induce short- and long-term DM because a longer survival of diabetic rats was only possible when a smaller dose of streptozotocin (30 vs 55 mg/kg body weight) was used. Nevertheless, this lower dose of streptozotocin was effective in inducing DM as shown by the constant fasting hyperglycemia in the chronic DM rats, which did not differ from the DM animals that received a larger streptozotocin dose, and by their marked decrease in body weight gain. The use of small doses of streptozotocin has been thought to induce an unstable diabetic state with large fasting blood glucose variations (15), but in our series the fasting hyperglycemia was relatively constant during the 6-month period, and the chronic diabetic rats had a good survival rate although they never received insulin.

The decrease in body weight after 6 and 15 days of DM was similar to that reported by other investigators studying periods ranging from 5 to 30 days after DM induction (2,16,17). Absolute thyroid and pituitary gland weights were unchanged in all 3 DM groups, in contrast to a significantly decreased thyroid or pituitary gland weight reported previously (17,18) for male Wistar rats 15 days after DM induction. This difference may be related to the different sex or strain of the animals studied.

Several investigators have reported decreased serum TSH as well as T₄ levels 10 to 30 days after DM induction (1-3,17,18). The decreased TSH has been ascribed to a diminished pituitary sensitivity to TRH (17,19) or to an intrinsic reduction in the constitutive and/or regulated TSH secretion by the diabetic thyrotropes (20). In the present study, we did not find significant changes in serum TSH of the diabetic rats at any of the times studied. Unaltered levels of serum TSH were also reported in short-term DM by Chopra et al. (5) and Moura et al. (4). After longer periods of DM (4 months), Tontis et al. (21) found serum TSH to be unchanged, although pituitary thyrotropes were markedly increased, suggesting that thyrotrone hyperplasia, compensatory for low thyroid hormone levels, may help to normalize plasma TSH, at least after 4 months of DM. However, it should be kept in mind that ‘normal’ serum TSH values when serum T4 is decreased, as observed in the short-term DM, indicate some impairment of TSH secretion.

Serum T₄ was decreased in the very short- and short-term DM rats, as reported by others (1-3,5), but not in the chronic DM rats. This indicates a change in the factors that determine the decreased T₄ secretion during the initial period of insulinoopenia. The study of Tontis et al. (21) seems to corroborate this, since the difference in serum T₄ between control and DM rats is markedly de-
increased 8 months after DM induction.

We were unable to determine serum T₃ in the present study, but a significant decrease in serum T₃ was found after 15 days of DM in a previous study using a similar experimental model (4), and decreased hepatic and renal type I iodothyronine-deiodinase activity in experimental DM has been well documented (5).

The significantly increased thyroid T₄-5'-deiodinase activity observed in the 15-day DM rats contrasts with our previous findings of an impaired thyroid peroxidase activity in the same experimental model (4), and shows that the changes produced by short-term insulinopenia cannot be explained by a diminished TSH stimulation of the thyroid gland. In diabetic mice, Bagchi et al. (22) found an increased cyclic AMP production in the thyroid, suggesting an increased sensitivity of the thyroid gland to TSH stimulation. It remains to be seen if a similar hypersensitivity may also be present in the thyroid of the short-term diabetic rat. The T₄-5'-deiodinase activity was not increased in the chronic DM rats.

Despite the marked decrease in serum T₄, total pituitary T₄-5'-deiodinase activity of 6- and 15-day DM rats showed no significant changes. Since only the total pituitary 5'-deiodination activity was determined in this study, we are unable to evaluate any specific change in type I or type II iodothyronine-deiodinase activities, which may occur in opposite directions and thus blunt any effect on total T₄ deiodination. A fact to be considered is that about 50% of the anterior pituitary gland is made up of somatotrophs (23). Thus, the pituitary deiodinase activity, as measured by the currently available methods, may not give a true picture of the real thyrotrope deiodinase activity.

After 6 months of DM, the pituitary DI activity was decreased, although serum T₄ and TSH and thyroid DI were within normal ranges. A decreased pituitary T₄-deiodination in the presence of normal T₄ should result in less pituitary T₃, and increased TSH secretion. This did not occur, and there was no difference in serum TSH levels between chronic DM and control rats. Thus, either the changes in the whole pituitary gland are not true estimates of thyrotrope deiodinase activity or long-standing diabetes produces some impairment of thyrotrope function.

Acknowledgments

We would like to thank Drs. Rui M.B. Maciel and Ewaldo M.K. Russo for the generous gift of T₄ antibody, and Mr. Advaldo N. Bezerra and Ms. Norma L.A. Faria for excellent technical assistance.

References


