Brazilian Journal of Medical and Biological Research (1997) 30: 269-274
ISSN 0100-879X

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

C.C.A. Nascimento-Saba[1,2], M.M.D. Breitenbach[2] and D. Rosenthal[1]

[1]Laboratório de Fisiologia Endócrina, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, 21491-900 Rio de Janeiro, RJ, Brasil
[2]Departamento de Ciências Fisiológicas, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, 20550-020 Rio de Janeiro, RJ, Brasil

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 T_4-5'-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 T_4, serum TSH and T_4-5'-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 T_4 as substrate. Mean serum T_4 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 T_4, even though pituitary T_4-5'-deiodinase activity was unchanged. Thyroid T_4-5'-deiodinase was unchanged after 6 days of diabetes mellitus, but was significantly increased from 20.6 to 37.0 pmol T_3/mg protein after 15 days. Six months after diabetes mellitus induction, both serum T_4 and thyroid T_4-5'-deiodinase returned to normal ranges and serum TSH was unchanged, although pituitary T_4-5'-deiodinase was now significantly decreased from 2.7 to 1.7 pmol T_3/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.

Key words
- Thyroid
- Pituitary
- Diabetes mellitus
- T_4-5'-deiodinase
- Serum T_4
- Serum TSH
- Rat
- Streptozotocin

Correspondence
D. Rosenthal
Laboratório de Fisiologia Endócrina
Instituto de Biofísica
Carlos Chagas Filho
CCS, UFRJ
21949-900 Rio de Janeiro, RJ
Brasil.
Fax: 55 (021) 280-8193
E-mail: doris@chagas.biof.ufrj.br

Research supported by CNPq (No. 530206/93.5), CEPG/UFRJ (SAG 350801P066-0) and FINEP (No. 6.6.92-0009.00).

We dedicate this paper to Prof. Carlos Chagas Filho, founder of the Institute of Biophysics, on the occasion of its 50th anniversary.

Received April 2, 1996
Accepted November 14, 1996

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, T_4, and T_3 (1-3), low thyroid peroxidase activity (4), as well as decreased hepatic and renal T_4-5'-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 T_4-5'-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 T_4-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 T_4-5'-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).

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. [a]N = 1.

Groups	N	ΔBody weight (g)	Thyroid (mg)	Pituitary (mg)	Blood glucose (mg/dl)
6 days					
DM	8	-18.2	13.4 ± 1.0	10.8 ± 1.0	319 ± 22
Control	8	4.1	13.5 ± 1.4	10.6 ± 1.6	120 ± 5
15 days					
DM	14	-21.8	11.5 ± 1.4	9.7 ± 2.0	328 ± 11
Control	14	9.2	11.6 ± 2.1	10.5 ± 2.6	112[a]
6 months					
DM	12	13.5	11.9 ± 3.2	10.2 ± 2.3	290 ± 34
Control	7	39.8	13.6 ± 0.9	11.6 ± 1.3	132 ± 12

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 T_4 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 250 mM sucrose and 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-

mined by the method of Bradford (11).

The assay mixture contained 100 µl microsome fraction and 2 µM T_4 in a final volume of 125 µl and was incubated at 37ºC for 20 min for the thyroid assay and for 60 min for the pituitary assay. The reaction was stopped by the addition of 250 µl ice-cold 95% ethanol. The T_3 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 T_3 formed per mg microsome fraction protein (pmol T_3/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 T_4 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).

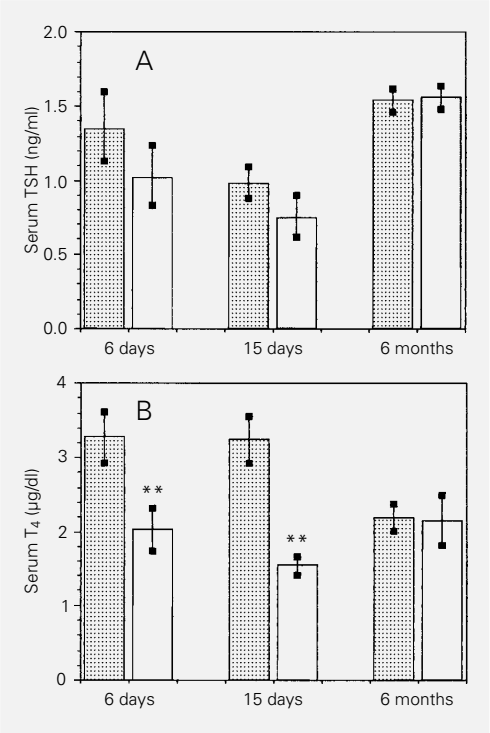

Figure 1 - Effect of very short-term (6 days), short-term (15 days) and chronic (6 months) streptozotocin-induced diabetes on rat serum TSH (A) and T_4 (B). Data are reported as mean ± SEM of control (▨) and diabetic rat groups (□). **P<0.01 compared to respective controls (Student-Newman-Keuls test). Number of experimental groups and doses of streptozotocin used are given in the legend to Table 1.

After 6 days of DM induction, neither pituitary DI (C: 1.38 ± 0.51; DM: 2.40 ± 0.74 pmol T_3/mg protein) nor thyroid DI (C: 13.58 ± 1.05; DM: 13.98 ± 1.71 pmol T_3/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 T_3/mg protein), whereas thyroid DI was significantly increased (C: 20.58 ± 3.78; DM: 37.04 ± 3.73 pmol T_3/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 T_3/mg protein) (Figure 2B), whereas pituitary DI was decreased (C: 2.74 ± 0.90; DM: 1.71 ± 0.44 pmol T_3/mg protein, P<0.05) (Figure 2A).

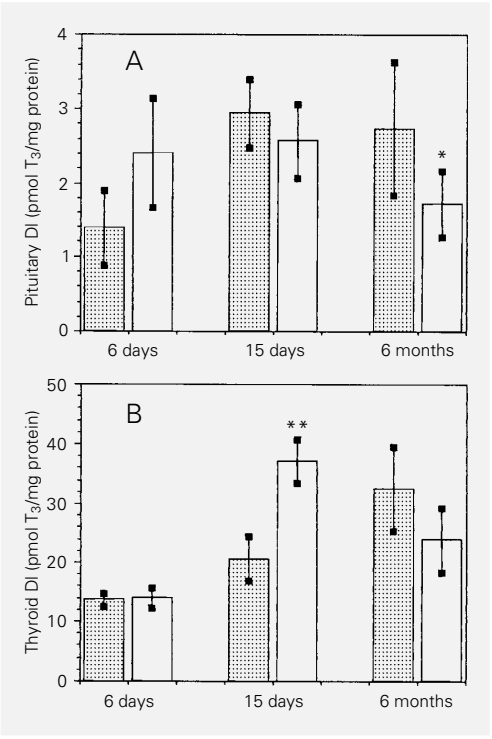

Figure 2 - Effect of very short-term (6 days), short-term (15 days) and chronic (6 months) streptozotocin-induced diabetes on rat pituitary (A) and thyroid (B) T_4-5′-deiodinase (DI) activity. Data are reported as mean ± SEM of control (▨) and diabetic rat groups (□). *P<0.05 and **P<0.01 compared to respective controls (Student-Newman-Keuls test). Number of experimental groups and doses of streptozotocin used are given in the legend to Table 1.

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 T_4 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 thyrotrope 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 T_4 is decreased, as observed in the short-term DM, indicate some impairment of TSH secretion.

Serum T_4 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 T_4 secretion during the initial period of insulinopenia. The study of Tontis et al. (21) seems to corroborate this, since the difference in serum T_4 between control and DM rats is markedly de-

creased 8 months after DM induction.

We were unable to determine serum T_3 in the present study, but a significant decrease in serum T_3 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 T_4-5'-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 T_4-5'-deiodinase activity was not increased in the chronic DM rats.

Despite the marked decrease in serum T_4, total pituitary T_4-5'-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 T_4 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 T_4 and TSH and thyroid DI were within normal ranges. A decreased pituitary T_4-deiodination in the presence of normal T_4 should result in less pituitary T_3, 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 T_4 antibody, and Mr. Advaldo N. Bezerra and Ms. Norma L.A. Faria for excellent technical assistance.

References

1. Mitsuma T & Nogimori T (1982). Effects of streptozotocin-induced diabetes mellitus on hypothalamic-pituitary-thyroid axis in rats. *Endocrinologica Japonica*, 29: 695-700.
2. Rondeel JMM, deGreef WJ, Heide R & Visser TJ (1992). Hypothalamo-hypophysial-thyroid axis in streptozotocin-induced diabetes. *Endocrinology*, 130: 216-220.
3. van der Elst JPS & van der Heide D (1992). Effects of streptozotocin-induced diabetes and food restriction on quantities and source of T_4 and T_3 in rat tissues. *Diabetes*, 41: 147-152.
4. Moura EG, Pazos CC & Rosenthal D (1986). Insulin deficiency impairs thyroid peroxidase activity. A study in experimental diabetes mellitus. In: Medeiros-Neto G & Gaitan E (Editors), *Frontiers in Endocrinology*. Plenum Medical Book Co., New York, 627-630.
5. Chopra IJ, Wiersinga W & Frank H (1981). Alterations in hepatic monodeiodination of iodothyronines in the diabetic rat. *Life Sciences*, 28: 1765-1776.
6. Ortiz-Caro J, Obregon MJ, Pascual A & Jolin T (1984). Decreased T_4 to T_3 conversion in tissues of streptozotocin-diabetic rats. *Acta Endocrinologica*, 106: 86-91.
7. Boye N & Laurberg P (1984). Deiodination of T_4 to T_3 and rT_3 by microsomes from normal human thyroid tissue. *Molecular and Cellular Endocrinology*, 37: 295-299.
8. Erickson VJ, Cavalieri RR & Rosenberg LL (1981). Phenolic and nonphenolic ring iodothyronine deiodinases from rat thyroid gland. *Endocrinology*, 108: 1257-1264.
9. Ishii H, Inada M, Tanaka K, Mashio Y, Naito K, Nishikawa M, Matsuzuka F, Kuma K & Imura H (1982). Sequential deiodination of thyroxine in human thyroid gland. *Journal of Clinical Endocrinology and Metabolism*, 55: 890-896.

10. Kaplan MM (1980). Thyroxine 5'-monodeiodination in rat anterior pituitary homogenates. *Endocrinology*, 106: 567-576.
11. Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.
12. Denardin OVP (1986). Influência do diabetes mellitus na conversão periférica de tiroxina (T_4) em 3,5,3'-triiodotironina (T_3): estudo com perfusão de fígado em ratos. Doctoral thesis, Escola Paulista de Medicina, São Paulo.
13. Zar JH (1974). *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, New Jersey.
14. Povoa LC (1980). Diabetes mellitus e outras endocrinopatias. In: Arduino F (Editor), *Diabetes mellitus*. Guanabara Koogan, Rio de Janeiro, 183-197.
15. Simon OR & West ME (1992). Unstable diabetic state produced by a small dose of streptozotocin in rats. *West Indian Medical Journal*, 42: 146-149.
16. Ferguson DC, Hoenig M & Jennings AS (1985). Triiodothyronine production by the perfused rat kidney is reduced by diabetes mellitus but not by fasting. *Endocrinology*, 117: 64-70.
17. Ortiz-Caro J, González C & Jolin T (1984). Diurnal variations of plasma growth hormone, thyrotropin, thyroxine and triiodothyronine in streptozotocin-diabetic and food-restricted rats. *Endocrinology*, 115: 2227-2232.
18. González C, Montoya E & Jolin T (1980). Effect of streptozotocin diabetes on the hypothalamic-pituitary-thyroid axis in the rat. *Endocrinology*, 107: 2099-2103.
19. Pastor RM & Jolin T (1983). Peripheral metabolism and secretion rate of thyrotropin in streptozotocin-diabetic rats. *Endocrinology*, 112: 1454-1459.
20. Bestetti GE, Brandli P & Rossi GL (1994). Secretory disorders in pituitary thyrotropes of streptozotocin-diabetic male rats. *Acta Anatomica*, 149: 215-220.
21. Tontis DK, Bestetti GE, Reymond MJ, Lemarchand-Béraud T & Rossi GL (1990). The age at onset of diabetes influences functional and structural changes in the pituitary-thyroid axis of streptozotocin-diabetic male rats. *Virchows Archiv B Cell Pathology*, 59: 205-213.
22. Bagchi N, Brown TR, Shivers B, Lucas S & Mack RE (1981). Decreased thyroidal response to thyrotropin in diabetic mice. *Endocrinology*, 109: 1428-1432.
23. Thorner MD, Vince ML, Horvarth E & Kovacs K (1992). The anterior pituitary. In: Wilson JD & Foster DW (Editors), *Williams' Textbook of Endocrinology*. 8th edn. W.B. Saunders, Philadelphia, 221-310.