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Autoradiographic thyroid evaluation in short-term experimental diabetes mellitus

C.C.A. Nascimento-Saba[1,3], A.C. Brito[1], M.J.S. Pereira[2], J.J. Carvalho[2] and D. Rosenthal[3]

Departamentos de [1]Ciências Fisiológicas and [2]Histologia e Embriologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil
[3]Laboratório de Fisiologia Endócrina, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

Abstract

Previous studies have shown that *in vitro* thyroid peroxidase (TPO) iodide oxidation activity is decreased and thyroid T4-5'-deiodinase activity is increased 15 days after induction of experimental diabetes mellitus (DM). In the present study we used thyroid histoautoradiography, an indirect assay of *in vivo* TPO activity, to determine the possible parallelism between the *in vitro* and *in vivo* changes induced by experimental DM. DM was induced in male Wistar rats (about 250 g body weight) by a single *ip* streptozotocin injection (45 mg/kg), while control (C) animals received a single injection of the vehicle. Seven and 30 days after diabetes induction, each diabetic and control animal was given *ip* a tracer dose of ^{125}I (2 µCi), 2.5 h before thyroid excision. The glands were counted, weighed, fixed in Bouin's solution, embedded in paraffin and cut. The sections were stained with HE and exposed to NTB-2 emulsion (Kodak). The autohistograms were developed and the quantitative distribution of silver grains was evaluated with a computerized image analyzer system. Thyroid radioiodine uptake was significantly decreased only after 30 days of DM (C: 0.38 ± 0.05 *vs* DM: 0.20 ± 0.04%/mg thyroid, $P<0.05$) while *in vivo* TPO activity was significantly decreased 7 and 30 days after DM induction (C: 5.3 and 4.5 grains/100 µm^2 *vs* DM: 2.9 and 1.6 grains/100 µm^2, respectively, $P<0.05$). These data suggest that insulin deficiency first reduces *in vivo* TPO activity during short-term experimental diabetes mellitus.

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

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Key words
- Thyroid
- Diabetes mellitus
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- Rat

Diabetes mellitus (DM) alters several aspects of thyroid gland function in humans and experimental animals. A decrease in radioiodine uptake and *in vitro* thyroid peroxidase (TPO) activity (1) and an increase in T4-5'-deiodinase activity have been reported in short-term streptozotocin-induced diabetic rats (2), as well as decreased serum thyroxin (T4) and triiodothyronine (T3) (1-3). The present study was undertaken to evaluate *in vivo* TPO activity estimated by thyroid autoradiography during short-term streptozotocin-induced DM.

Male Wistar rats, aged 3 months, were maintained in a temperature-controlled room (25°C) on a light/dark cycle of 12/12 h and

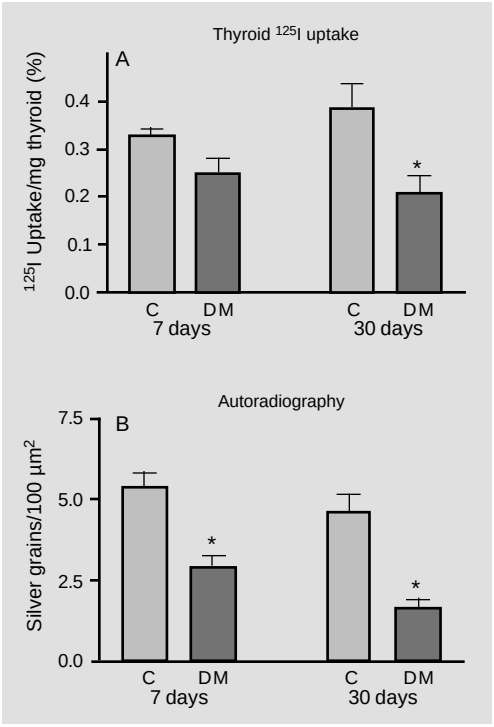

Figure 1 - Effect of streptozotocin-induced diabetes mellitus on rat thyroid ^{125}I uptake (A) and silver grain distribution (B) of control (C) and diabetic (DM) rats after 7 and 30 days of experimental diabetes. Data are reported as mean ± SEM for 5 animals per group. *P<0.05 compared to the respective controls (Student-Newman-Keuls test).

received commercial pellet chow (Purina) and water *ad libitum*. Diabetes mellitus was induced by a single *ip* injection of streptozotocin (45 mg/kg body weight; Sigma Chemical Co., St. Louis, MO) dissolved in 0.5 ml 50 mM citrate buffer, pH 4.5. 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 experiment using a Glucofilm Glucometer (Ames, Elkart, IN). Insulin was not administered to any of the diabetic animals.

Seven and 30 days after diabetes induction, diabetic and control rats received a tracer dose of ^{125}I (2 µCi) *ip*, and 2.5 h later they were anesthetized with ether. Thyroid glands were rapidly removed and weighed and thyroid radioiodine uptake was individually determined with a gamma counter (Cobra Auto-gamma, Packard Instrument Co., Downers Grove, IL). The thyroids were then fixed in Bouin's solution, embedded in paraffin and cut into 5-µm sections. The sections were mounted on glass slides, stained with HE and covered with NTB-2 emulsion (Kodak). After 7 days of exposure at 4ºC they were developed with Kodak D 19b developer (4). The quantitative distribution of the silver grains was determined with a light microscope (100X objective lens) and analyzed with a computerized image analyzer software system (Image pro plus, Media Cibernetics, Silver Spring, MA), using a computer grid of 25 meshes per 100 µm^2. Data are reported as means ± SEM, and ANOVA was used for statistical evaluation, with the level of significance set at $P<0.05$.

The DM animals had a significant body weight loss during the experimental period, while the thyroid gland weights were not significantly different after 7 days (C: 13.7 ± 1.2 mg *vs* DM: 13.3 ± 0.7 mg) or 30 days of DM (C: 15.2 ± 1.1 mg *vs* DM: 14.4 ± 1.0 mg). Plasma glucose levels of both DM groups were significantly increased by two- or three-fold in relation to controls (C: 184.8 ± 19.9 and 141.4 ± 16.7 mg/dl *vs* DM: 285.8 ± 34.2 and 360 ± 38 mg/dl, after 7 and 30 days, respectively). Thyroid radioiodine uptake was significantly decreased in DM animals only after 30 days (Figure 1A). However, the silver grain distribution was already significantly decreased after 7 days and even more after 30 days of diabetes induction (Figure 1B). Figure 2 shows the grid utilized for silver grain quantification and the difference between control (A) and diabetic rats (B) after 7 days of DM.

It is well known that the lack of insulin can affect thyroid function but the mechanisms that cause this dysfunction are still unknown. Fasting hyperglycemia (above 250 mg/dl) was the determinant of diabetes 48 h after streptozotocin injection. The diminished body weight of diabetic animals has already been reported (1,2,5,6), while un-

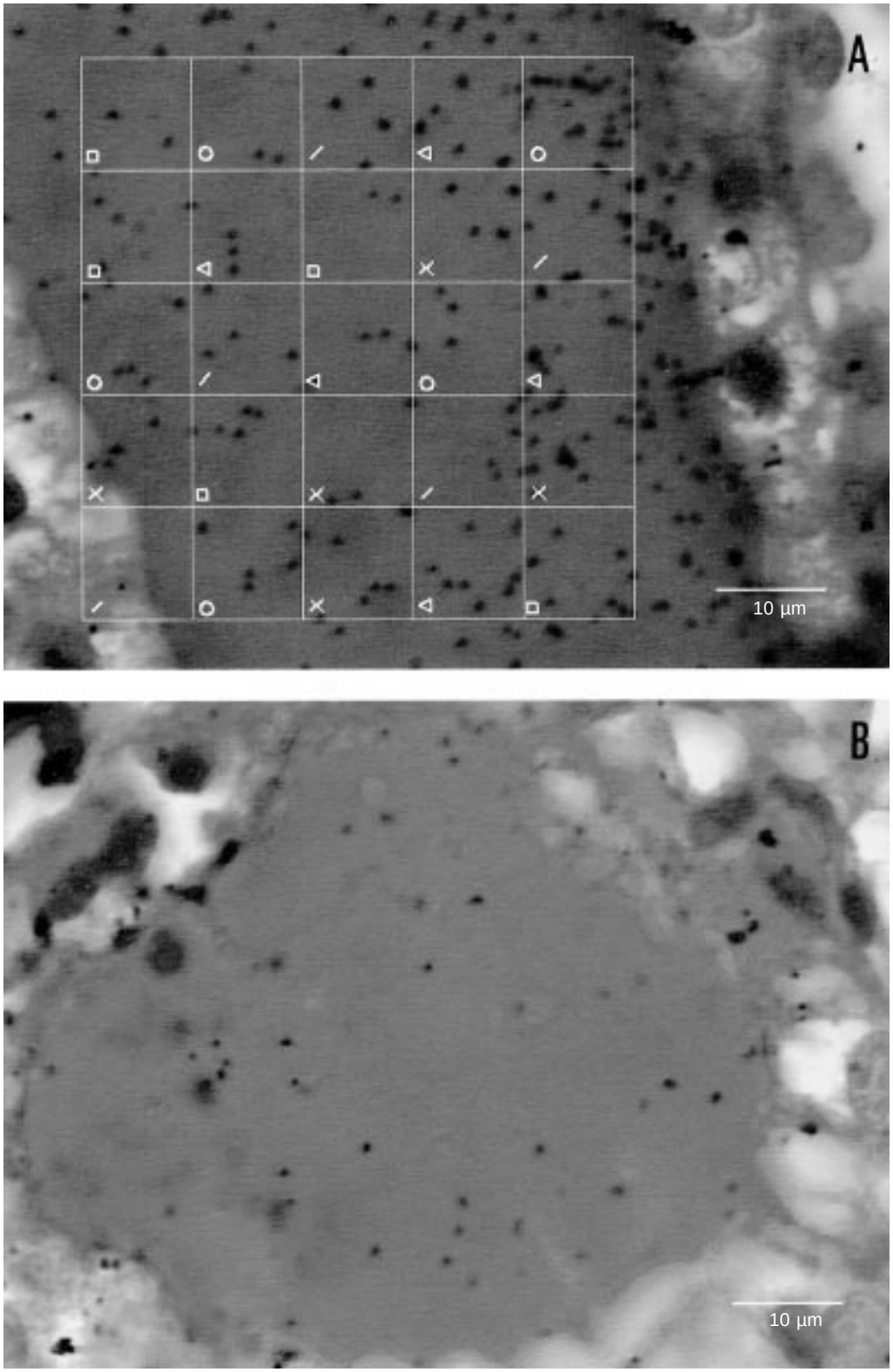

Figure 2 - *A*, Autoradiograph of a normal rat thyroid taken from a 3-month-old animal and labeled with ^{125}I for 2.5 h. The 25-mesh computer grid utilized to quantitate the silver grains is shown. *B*, Autoradiograph of a streptozotocin-induced diabetic rat thyroid taken from a 3-month-old animal 7 days after DM induction. HE stained. Bar = 10 µm.

changed thyroid gland weights confirm our previous reports (2), although they disagree with the findings of others (6). Our studies are the first to show that iodide uptake at the thyrocyte basement membrane is impaired and the efficiency of radioiodine organification by thyroid peroxidase is also decreased *in vivo* after 7 days of DM. Thus, our data show that the effects of diabetes mellitus or insulinopenia on *in vivo* TPO activity appear sooner than suggested in previous studies (1). Furthermore, it also seems that *in vivo* TPO activity is already affected at a time when thyroid iodide uptake is not yet significantly impaired.

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