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Short communication

Thyroxine induces pancreatic beta-cell apoptosis in rats

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Abstract

Aims/hypothesis. Thyroid hormones reduce glucose tolerance in animals and humans. This effect is accompanied by a reduction in the beta-cell volume of the pancreas.
Methods. We studied the underlying mechanism using terminal UTP nick end labelling (TUNEL) and caspase-3 to analyse apoptosis and BrdU labelling of beta-cell proliferation.
Results. The reason for the reduction of the beta-cell volume of the pancreas after thyroxine treatment is apparently an increased rate of beta-cell apoptosis by an increase of TUNEL and caspase-3 positive rat beta cells. In parallel, thyroxine treatment increased the rate of apoptosis in rat pancreatic ductal cells which are considered to contribute to the pool of stem cells from which insulin-producing beta cells originate. The effects of thyroid hormone treatment are reversible through an increase of the beta-cell replication rate when thyroxine is withdrawn as documented by an increase of the BrdU labelling index.
Conclusion/interpretation. An increased rate of beta-cell death due to apoptosis causes a decrease of insulin content and glucose-induced insulin secretion from the pancreas in hyperthyroidism. The resulting reduction of beta cells in the pancreas can provide an explanation for the decrease of glucose tolerance in hyperthyroidism. [Diabetologia (2002) 45:851–855]

Keywords Beta cell, Thyroxine, Apoptosis, Proliferation.

Thyroid hormones have a multitude of metabolic effects [1] and act as insulin antagonists [2]. Hyperthyroidism induces glucose intolerance in animals and humans [2, 3]. In particularly prone individuals, hyperthyroidism can induce a so-called “thyroid diabetes” [2]. Thyroxine treatment causes a state of experimental hyperthyroidism and reduces the pancreatic insulin content and glucose-induced insulin secretion [4, 5]. This is accompanied by a reduction in the beta-cell volume of the pancreas [5]. However, the mechanism underlying pancreatic beta-cell loss is not known [2]. The prohormone thyroxine is deiodinated to triiodothyronine in many tissues [6] including pancreatic islets [7]. Triiodothyronine mediates its effects through binding to thyroid hormone nuclear receptors [6]. As thyroid hormones play a major role in apoptotic tissue remodelling [8] we studied whether the effects of thyroxine on the endocrine pancreas might be caused by an increased rate of apoptosis of the beta cells in the islets of Langerhans.

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Abbreviations: TUNEL, TdT-mediated dUTP-x-nick end labelling; BrdU, 5-bromo-2' -deoxyuridine

Materials and methods

Experimental design. Experimental hyperthyroidism accompanied by mild hyperglycaemia (5.5 ± 0.1 vs 4.7 ± 0.1 mmol/l in control rats; $p < 0.05$, Student’s t test) and mild hyperinsulinaemia (0.59 ± 0.11 vs. 0.40 ± 0.04 ng/ml in control rats; $p < 0.05$, Student’s t test) was induced in Lewis rats (190–230 g) through treatment with L-thyroxine (600 µg/kg

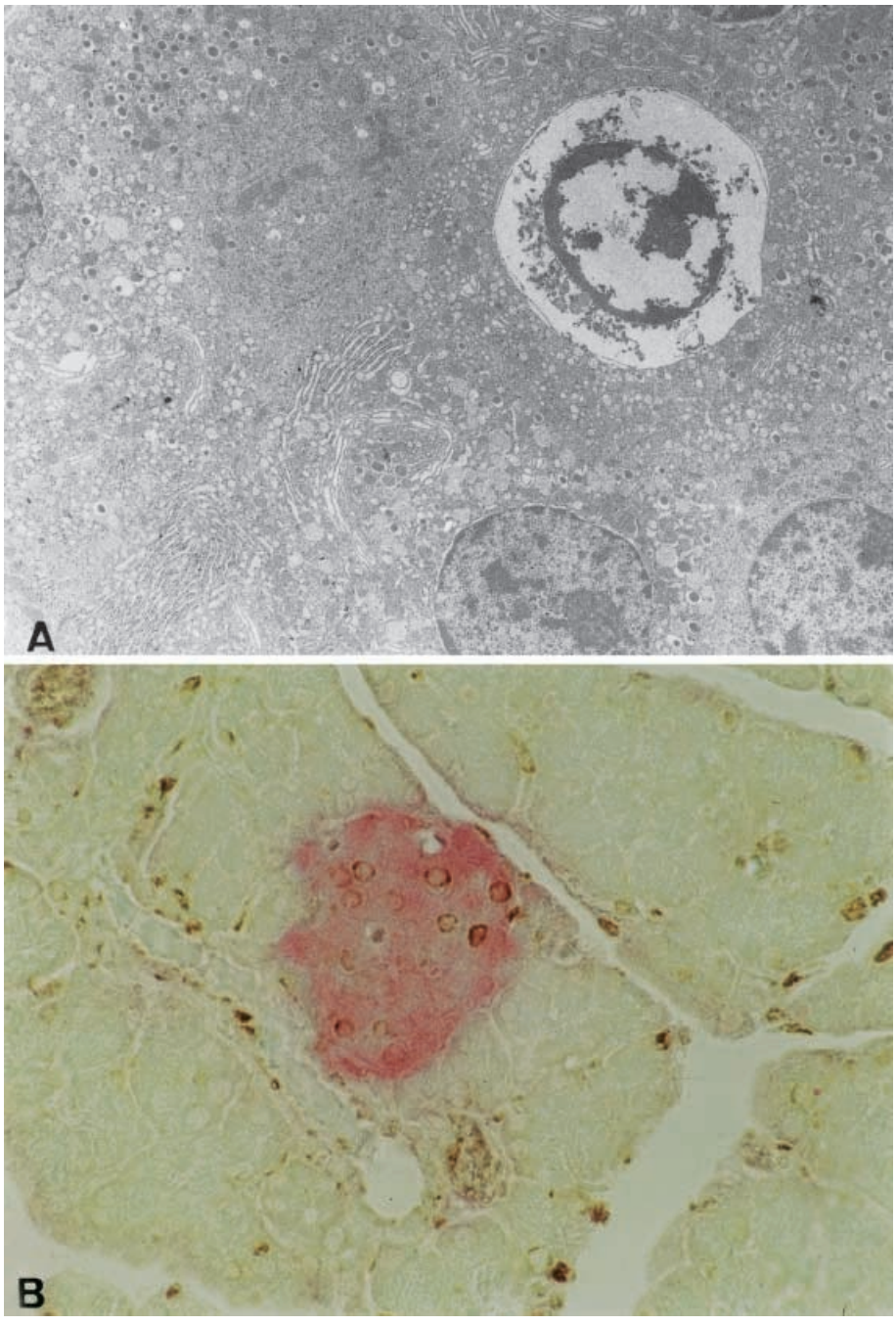

Fig. 1 A, B. Effects of thyroxine treatment (20 mg/kg b.wt.) upon apoptosis and proliferation of pancreatic beta cells. **A** shows the ultrastructure of an apoptotic beta cell surrounded by intact islet cells in an islet from a rat 3 days after administration (×5600) and **B** shows an immunostaining of proliferating beta cells identified through BrdU staining (100 mg/kg b.wt. injected i.p. 4 h before the experiment) of the nucleus (brown) and insulin staining of the cytoplasm (red) in an islet from a rat 5 days after administration (×620)

b.wt. given i.p. for 5 consecutive days) (Sigma, St. Louis, Mo., USA) dissolved in 0.9% NaCl solution [4, 9]. Alternatively, in time course studies, L-thyroxine (20 mg/kg b.wt.) was given as a single i.p. dose at different time points before the experiment, also using an established treatment schedule [10]. Control animals received a solution of 0.9% NaCl. On the day of the experiment the pancreas was removed from the animals fasted for 24 h and fixed for morphological analyses. Four hours before the experiment all animals received an i.p. injection of 5-bromo-2′-deoxyuridine (BrdU) (100 mg/kg b.wt.) (Sigma). For immunocytochemistry and electron microscopy, tissue specimens were processed and stained [11]. The animal experiments were carried out according to the specific national laws applicable in Germany under a license for animal experimentation granted by the local government of Hannover to S. Lenzen.

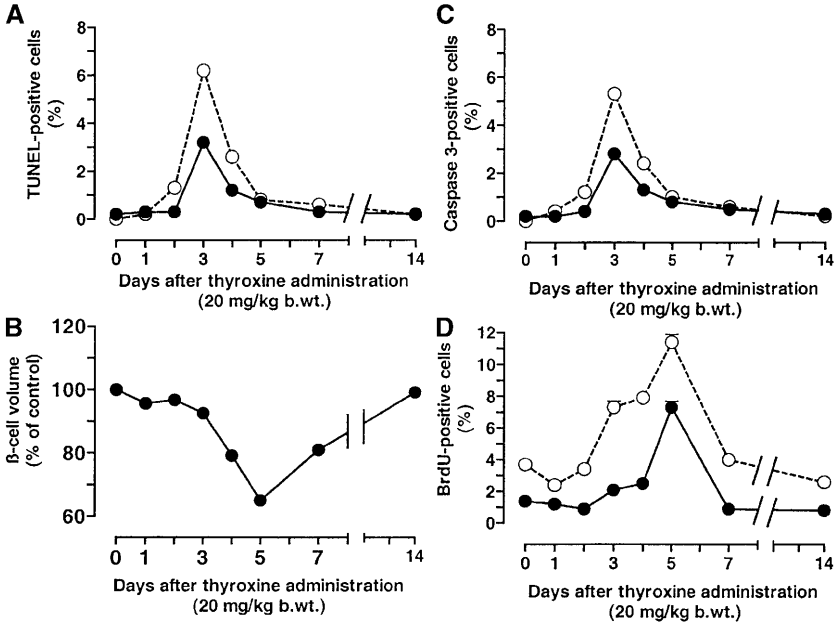

Fig. 2 A–D. Time-dependent effect of thyroxine treatment (20 mg/kg b.wt.) upon the rate of apoptosis (**A** and **B**) as well as the beta-cell volume of the pancreas (**C**) and the proliferation rate (**D**) in rat pancreatic beta cells (●) and rat pancreatic ductal cells (○). **A** TUNEL assay; **B** caspase-3 staining; **C** beta-cell volume of the pancreas (control 100% value was 1.14 ± 0.37 mm^3/pancreas); **D** BrdU labelling. Shown are means ± SEM of four experiments. SEM are depicted only when larger than the symbols

Apoptosis and proliferation. Apoptotic cells of islets and pancreatic ducts with a circumference greater than 10 cells were identified using a terminal UTP nick end labelling (TUNEL) cell death detection kit (Roche, Mannheim, Germany) and activated caspase-3 in the cytoplasm (Pharmingen, Hamburg, Germany). Proliferating cells were stained using a BrdU staining kit (Zytomed, Berlin, Germany). Positively stained nuclei were expressed either as percent of the total number of nuclei from beta cells in the islets immunostained for insulin (Novo, Bagsvaerd, Denmark) or as percent of the total number of ductal cells immunostained for cytokeratin 20 (DAKO, Hamburg, Germany). The sections were quantified morphometrically using a computer-assisted method [12]. The relative beta-cell volume was obtained by calculating the ratio between the area occupied by the insulin positive cells and the area of the total tissue section. The total beta-cell volume per pancreas was obtained by multiplying this ratio by the total weight of the pancreas divided by its density [12].

Statistical analyses. Data are expressed as mean values ± SEM and tested for statistical significance using Student's *t* test. A *p* value of less than 0.05 was considered significant.

Results

When rats (n = 4) were treated with thyroxine (600 µg/kg b.wt. given i.p. for 5 consecutive days) the parameters of beta-cell and ductal-cell apoptosis in the rat pancreas increased. The percentage of TUNEL positive beta cells increased from 0.2 ± 0.1% in control rats to 1.6 ± 0.2% ($p < 0.05$) in thyroxine treated rats and the percentage of caspase-3 positive beta cells increased from 0.2 ± 0.1% to 1.5 ± 0.2% ($p < 0.05$, Student's t test). This thyroxine-induced beta-cell apoptosis resulted in a 24.8 ± 1.2% reduction of the total islet area per pancreas section.

The percentage of TUNEL positive ductal cells increased from 0.2 ± 0.1% in control rats to 1.9 ± 0.2% ($p < 0.05$) in thyroxine treated rats and the percentage of caspase-3 positive ductal cells increased from 0.2 ± 0.1% to 1.7 ± 0.2% ($p < 0.05$).

The diagnosis of apoptosis was confirmed through electron microscopy by the typical signs of margination and condensation of the nuclear chromatin in a pancreatic islet section (Fig. 1A).

The decrease of the beta-cell volume induced by apoptosis can explain the reduction of insulin content in the whole pancreas and glucose-induced insulin secretion from the pancreas of rats treated with this thyroxine treatment schedule [5, 13].

Through application of a single dose of thyroxine (20 mg/kg b.wt. given i.p.) it was possible to synchronise the process of apoptosis. With this treatment schedule a time-dependent process with the maximal rate of beta-cell apoptosis in the rat pancreas at the third day after thyroxine administration could be depicted (Fig. 2). This could be shown by an increase of the TUNEL positive as well as of the caspase-3 positive pancreatic beta cells (Fig. 2A, B). In parallel with the increased rate of beta cells, the apoptosis rate increased also in rat pancreatic ductal cells (Fig. 2A, B).

Again, in parallel these effects of thyroxine were accompanied by a time-dependent decrease of the beta-cell volume of the pancreas which became maximal 5 days after thyroxine administration (Fig. 2C). This

can explain the reduction of glucose-induced insulin secretion caused in rats treated with this thyroxine treatment schedule.

About 5 to 7 days after the application of thyroxine, the rate of apoptosis of beta cells and ductal cells had returned to basal again and remained low even 14 days after the hormone application (Fig. 2A, B).

The reduction of the beta-cell volume of the pancreas after thyroxine administration was reversible (Fig. 2C). Already 7 days after thyroxine administration the beta-cell volume of the pancreas had increased again and it was fully restored after 14 days (Fig. 2C). The explanation for this normalisation of the beta-cell volume was a large increase of the beta-cell replication rate (Fig. 1B). This was documented by the increase of the BrdU labelling index which followed the increase of the apoptosis rate with a 2-day delay as shown by the maximum rate of beta-cell replication at day 5 after thyroxine treatment (Fig. 2D). The parallel increase of the BrdU labelling index for the ductal cells (Fig. 2D) shows that the loss of pancreatic ductal cells by thyroxine treatment was compensated also in this cell type through replication.

Other pancreatic islet cell types, in particular α-cells and δ-cells as well as the exocrine pancreas, did not react to thyroxine treatment with an increased rate of apoptosis as shown by electron microscopy. On morphological examination no signs of inflammation were observed in the rat pancreas indicating that thyroxine-induced apoptosis is not accompanied by immunological phenomena, at variance from the situation typically observed in animal models of Type I (insulin-dependent) diabetes mellitus [14, 15].

Discussion

In this study thyroxine treatment of rats reduced glucose tolerance confirming earlier results [2]. This treatment increased the rate of apoptosis in pancreatic beta cells as shown by an increase of TUNEL and caspase-3 positive cells. Of interest, thyroxine treatment increased the rate of apoptosis also in pancreatic ductal cells which are considered to contribute to the pool of stem cells from which insulin-producing beta cells originate [16, 17]. Thyroid hormones possibly induce apoptosis in pancreatic beta cells through the activation of cell-death pathways. This could be achieved by affecting gene transcription through binding to nuclear thyroid hormone receptors even if the exact mechanisms underlying thyroid hormone-induced apoptosis are still not known [6, 8]. Considering that the birth rate of new beta cells in the rodent pancreas has been calculated to be 3% new cells per day [17], an antagonistic apoptotic action of thyroid hormones could help maintain a physiological balance between beta-cell replication and beta-cell death in the endocrine pancreas.

The increased loss of beta cells from the endocrine pancreas due to thyroid hormone-induced apoptosis in the hyperthyroid state puts an increased demand on each single remaining beta cell, which have to compensate for the increased insulin requirements due to thyroxine-induced peripheral insulin resistance [2]. An increase of the blood glucose concentration which typically accompanies the hyperthyroid state [2, 3] can help meet the insulin demands through additional mobilisation of insulin from the remaining beta cells. However, exhaustion of the insulin releasing capacity, after a period of overstimulation, is more likely to emerge, typically resulting in an insulin secretory failure which could manifest in the development of overt “thyroid diabetes” [2].

Thus medical efforts should be directed towards prevention of over-challenging the insulin secretory reserve of the endocrine pancreas such as in hyperthyroidism or during exogenous thyroid hormone administration [2]. This could be particularly important in people with a limited endogenous insulin secretory capacity who are most susceptible to the development of “thyroid diabetes” such as elderly patients with a borderline insulin secretory capacity [2]. This should be kept in mind also when, for therapeutic reasons, thyroid hormone supplementation is considered.

Thus an increased rate of apoptosis is probably responsible for the thyroid hormone-induced reduction in the beta-cell volume of the pancreas which is associated with a decrease of glucose-induced insulin secretion in hyperthyroidism [5]. The apoptosis-induced reduction in the beta-cell volume of the pancreas can also provide an explanation for the decrease of glucose tolerance in hyperthyroidism and the development of “thyroid diabetes”, representing a form of Type II (non-insulin-dependent) diabetes mellitus, in animals and in humans already with a low beta-cell mass or pre-existing evidence of impaired beta-cell function [2].

On the other hand, our results show that the effects of thyroid hormones are reversible through an increase of the beta-cell replication rate when the hormone effect fades away as documented by the increase of the BrdU labelling index. This is an observation of clinical relevance since it shows that a reduction of the thyroid hormone effect is apparently accompanied by a reduction in the rate of beta-cell apoptosis. It also explains why antithyroid therapy is able to increase the insulin secretory capacity of the pancreas and to improve impaired glucose tolerance [2].

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