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Triiodothyronine (T_3)-mediated toxicity and induction of apoptosis in insulin-producing INS-1 cells

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Abstract

Thyroid hormones reduce glucose tolerance in humans and animals. This effect is related to a decrease of glucose-induced insulin secretion following a reduction of pancreatic beta cell mass due to beta cell loss. The aim of this study was to analyze in vitro the mechanisms underlying the effects of triiodothyronine (T_3) on the cell viability and cell cycle caused by changes of cell death or proliferation rate of insulin-producing INS-1 cells. 72-h Exposure of INS-1 cells to increasing T_3 concentrations up to 500 μM resulted in a significant viability reduction. This T_3 toxicity was caused by an increased apoptotic cell death rate, which was accompanied by a decreased proliferation rate. Inhibitory effects of T_3 on glucose-induced insulin secretion were already seen after 24 h of incubation, indicating that the deleterious effects of T_3 were time-dependent, changing from specific cellular dysfunctions to a severe and extended disturbance of the cellular survival program. Only T_3 concentrations higher than 250 μM were able to decrease cell viability and proliferation rate, to increase the rate of apoptosis and to reduce glucose-induced insulin secretion. These micromolar T_3 concentrations were significantly higher than the concentration range of T_3 receptor binding, indicating that other non-receptor-mediated mechanisms beyond the receptor level must be responsible for the observed toxic effects of T_3 in vitro.

Keywords: Insulin-producing cells; Thyroid hormones; Receptors; Cell death

Introduction

Thyroid hormones reduce glucose tolerance in humans and animals (Lenzen and Bailey, 1984). This effect is accompanied by a decrease of glucose-induced insulin secretion from the pancreas (Lenzen et al., 1976) and a reduced pancreatic beta cell mass (Lenzen and Klöppel, 1978). These thyroid hormone effects on glucose homeostasis and on endocrine pancreas function are apparently the result of an interaction of the hormone with its receptors (Yen, 2001).

Thyroid hormone receptors (TRs) are members of the nuclear receptor superfamily that comprises for steroid hormones, peroxisomal proliferator-activated receptor ligands and retinoids. Apart from the TRα2 and TRα3 isoforms, lacking the triiodothyronine (T_3) binding domain, all other TR isoforms have been shown to bind T_3 with a high affinity, indicated by their reported K_d-values in the range between 10^{-10} and 10^{-11} M (Lazar and Chin, 1990).

Although the exact function and physiological role of T_3 could be elucidated in some detail in the past, the importance of the T_3 action for the physiological function of insulin-producing cells and especially for the T_3-induced toxicity in pancreatic beta cells is still unknown. The aim of the present study was to analyze whether T_3 shows in vitro the same cytotoxic effects on insulin-producing cells as has been shown in vivo for beta cells (Jörns et al., 2002) and whether the observed functional changes induced by T_3 are dependent or independent from TR-mediated signal transduction.

Materials and methods

Materials

Triiodothyronine (3,3′,5-triiodo-L-thyronine; sodium salt) (T_3) was purchased from Fluka (Munich, Germany). All tissue

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culture equipment was purchased from Invitrogen (Karlsruhe, Germany). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), the DNA binding dyes propidium iodide and Hoechst 33342 and all other reagents were from Merck (Darmstadt, Germany).

Tissue culture of insulin-producing cells

Rat insulin-producing INS-1 tissue culture cells (Asfari et al., 1992) were cultured in RPMI 1640 tissue culture medium, supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 50 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere at 37 °C and 5% CO_2.

Exposure to triiodothyronine

T_3 (3,3′,5-triiodo-L-thyronine) was dissolved in 1 M NaOH, brought to a final concentration of 10^{-2} M with RPMI medium and filtered through a 0.22-μm filter. This T_3 stock solution was diluted in the culture medium immediately before use. The same amount of NaOH was added to the medium of the control incubations, which did not affect any of the variables evaluated. The cells were exposed to different concentrations of T_3 for different time periods. Camptothecin (1 μM) was used as a positive control for apoptosis-induced cell death.

Determination of cell viability and proliferation rate

INS-1 cells were seeded at a concentration of 5×10^4 cells/well in 100 μl culture medium in 96-well microplates and allowed to attach for a period of 24 h before they were incubated with 100, 250 or 500 μM T_3. After 24, 48 or 72 h of incubation, the viability of the cells was determined using a microtiter plate-based MTT assay (Mosmann, 1983). Viability was expressed as percent of the untreated samples. In order to study the effect of T_3 on the proliferation rate, the same concentrations of the hormone were used for a 72-h incubation period. The proliferation rate of the cells was quantified by the colorimetric BrdU (5-bromo-2′-deoxyuridine) Cell Proliferation ELISA (Roche Molecular Biochemicals, Mannheim, Germany) and the data were expressed in percent of untreated cells after 72-h exposure to T_3 as described earlier (Lortz et al., 2005).

Measurement of caspase-3 activity

Cells were exposed to 100, 250 or 500 μM of T_3 and after 6, 12, 24, or 48 h they were lysed with a lysis buffer (50 mM HEPES, 0.1% CHAPS, 5 mM DTT, 0.1 mM EDTA at pH 7.4), centrifuged at 10,000 rpm for 10 min, and the supernatant was stored at −70 °C until the day of the assay. Caspase-3 activity was determined with a luminescent caspase assay system (Promega) in 100 μl of reaction mixture containing 50 μg of protein and acetyl-DEVD-*p*-nitroanilide as a substrate. The luminescence was measured in a multilabel counter (Wallac 1420 Victor^2, Perkin Elmer LAS, Rodgau, Germany). The protein content of the supernatant was determined using the Bradford assay.

Quantification of apoptosis and necrosis by differential fluorescence staining

The percentage of apoptotic and necrotic cells was determined after 24- to 72-h exposure to 100, 250 or 500 μM of T_3 or 1 μM of camptothecin. Cells were incubated for 15 min with the DNA binding dyes propidium iodide (PI, 10 μg/ml) and Hoechst 33342 (HO 342, 20 μg/ml) (Delaney et al., 1997). Image acquisition was performed with an Olympus IX81 inverted microscope (Olympus Optical Co., Tokyo, Japan) equipped with a cell^R MT20 illumination system (Olympus BioSystems, Planegg, Germany) with UV excitation at 340–380 nm. Viable cells were identified by their intact nuclei with blue fluorescence (HO 342), necrotic cells by their intact nuclei with yellow–red fluorescence (HO 342 plus PI), and apoptotic cells by their fragmented nuclei, exhibiting either a blue (HO 342, early apoptosis) or yellow–red fluorescence (HO 342 plus PI, late apoptosis).

Ultrastructural characterization of cell viability and integrity

For electron microscopy, cell pellets of T_3-treated cells were fixed in 2% para-formaldehyde and 2% glutaraldehyde, in 0.1 M cacodylate buffer, pH 7.3, postfixed in 1% OsO_4 and finally embedded in Epon. Thin sections were contrast-stained with saturated solutions of lead citrate and uranyl acetate and viewed in an electron microscope (Gurgul et al., 2004).

Insulin secretion

Cells were seeded at a density of 250,000 cells/well in 6-well culture dishes and grown in RPMI 1640 medium supplemented with 10 mmol/l glucose. After 24 h cells were exposed for 24 h to 0, 100, 250 or 500 μM of T_3. Cells were then washed twice with glucose-free medium and pre-incubated with glucose-free medium for 120 min at 37 °C. Thereafter the rate of insulin secretion was measured during a 60-min incubation period in Krebs–Ringer buffer containing 0 or 10 mM glucose. After incubation, buffer was collected and gently centrifuged to remove detached cells. Insulin was determined radioimmunologically after appropriate dilution using a rat insulin standard. Polyethylene glycol was used to precipitate antibody-bound insulin (Tiedge et al., 2000). Insulin secretion values were normalized to the DNA content of the incubated cells, determined fluorimetrically using the double-stranded DNA binding dye *PicoGreen* (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions.

Statistics analysis

Data are expressed as means ± SEM. Unless stated otherwise, statistical analyses were performed using ANOVA plus Bonferroni test for multiple comparisons. Half-maximal effective concentrations (EC_{50}) were calculated from nonlinear regression analyses using least square algorithms of the Prism analysis program (Graphpad, San Diego, CA).