CLINICAL STUDY

Plasma thyrotropin bioactivity in Down's syndrome children with subclinical hypothyroidism

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Abstract

Objective: Subclinical hypothyroidism occurs in a number of children with Down's syndrome (DS). The reason for the mildly elevated plasma thyrotropin (TSH) concentrations is not known. The present study investigated whether decreased TSH bioactivity plays a role in this phenomenon.

Design: A retrospective study of plasma specimens from DS children with mildly elevated plasma TSH concentrations and thyroid hormone levels within the reference range, using a TSH receptor–adenylate cyclase mediated bioassay.

Methods: Strain JP26 Chinese hamster ovary (CHO) cells, stable transfected with the human TSH receptor, were incubated with unfractionated plasma (1/10 diluted in hypotonic incubation medium) of 10 DS children with subclinical hypothyroidism and nine euthyroid children with insulin-dependent diabetes mellitus as controls. cAMP released in the incubation medium was measured by RIA. Mock-transfected CHO cells were used to correct for non-specific CHO response. WHO Second International Reference Preparation of human TSH was dissolved and diluted in pooled normal human plasma and simultaneously bioassayed to match patient and control results.

Results: Plasma TSH levels were slightly increased in DS (mean $\pm$ s.d., 6.5 ± 1.3 mU/l, reference range 0.4–4.0 mU/l). Plasma TSH levels for controls (1.3 ± 0.4 mU/l) were within the reference range. Plasma thyroid hormone levels in patients and controls were normal, plasma TSH binding inhibitory immunoglobulin and thyroid peroxidase antibodies were negative. cAMP levels (corrected for non-specific CHO response) in DS patients (18.4 ± 3.9 pmol/well) and in controls (14.3 ± 1.3 pmol/well) did not significantly differ from cAMP levels generated by patient-TSH equivalent TSH standards (16.3 ± 0.9 pmol/well).

Conclusions: The present results demonstrate normal TSH bioactivity in plasma of DS children, indicating that subclinical hypothyroidism in these patients is of primary (thyroidal) origin.

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Introduction

Thyroid dysfunction is highly prevalent in Down's syndrome (DS) (1–3). Although overt hypothyroidism caused by thyroid autoimmunity is the best-known thyroid problem in DS, mildly elevated plasma thyrotropin (TSH) concentrations in the absence of signs of thyroid autoimmunity are seen much more frequently, especially in (young) DS children. The cause of this phenomenon, also known as subclinical hypothyroidism, is not known and is a common therapeutic dilemma (2).

Pituitary TSH is produced and secreted in many glycosylation forms that differ in carbohydrate content and branching, sialylation and sulfation. These variations have great influence on the metabolic clearance

rate and *in vitro* bioactivity of TSH (4–6). Several studies have shown that TSH bioactivity can be enhanced or reduced depending on the physiological condition, thyroidal or non-thyroidal disease (7–9). The availability of a bioassay using recombinant human TSH receptor (TSH-R) expressed in Chinese hamster ovary (CHO) cells, in which the TSH-mediated production of cAMP can be measured (10), greatly facilitates such studies.

In order to gain further insight into the cause of the isolated mild TSH elevation, we measured TSH bioactivity in unfractionated plasma in DS patients with subclinical hypothyroidism. As a control, TSH bioactivity was measured in plasma of euthyroid insulin-dependent diabetes mellitus (IDDM) children with TSH levels within the reference range.

Subjects and methods

Subjects

This study included ten children with DS (three males, seven females) aged 1.5–13 years, who were regularly seen in our outpatient clinic. All patients had subclinical hypothyroidism (elevated plasma TSH concentration and free (F) thyroxine (T_4) levels between 14.3 and 18.5 pmol/l; reference range 10–23 pmol/l) in the absence of signs of autoimmunity. None of the children was treated with thyroid hormone at the time of blood collection. In case of thyroid hormone treatment previously, treatment was discontinued 4 weeks before blood collection. IDDM controls (four males, five females) aged 2–17 years were euthyroid with plasma TSH, T_4 and FT_4 within the reference range and showed no signs of thyroid autoimmunity.

Samples

Heparin anticoagulated venous blood samples were collected from all individuals and stored at -20°C until assay. The Second International Reference Preparation 80/558 of TSH, human (IRP-TSH, WHO, London, UK) (11) was dissolved and serially diluted with pooled normal human plasma (TSH = 1.8 mU/l) to obtain appropriate standards ('standard plasma').

Cell culture

Chinese hamster ovary (CHO) cells stable transfected with the human TSH-R cDNA and the neomycin resistance gene (CHO-JP26 cells) or with the neomycin resistance gene alone (JP0 cells) were used in this study. The cells were kindly supplied by Dr G Vassart, IRIBHN, Université Libre de Bruxelles, Brussels, Belgium. Cells were cultured in RPMI 1640 (GIBCO Laboratories, Grand Island, NE, USA) supplemented with 1 mmol/l glutamine, antibiotics, 10% fetal calf serum (FCS) and 400 mg/l geneticin (Boehringer Mannheim, Almere, The Netherlands) in 5% CO_2 at 37°C in a humidified incubator.

Bioassay

The bioassay was carried out according to Persani *et al.* (10) with some modifications.

In brief, 30 000 to 40 000 cells in 100 μl RPMI (10% FCS, without geneticin) were seeded into 96-well plates. After 5 h culture, 100 μl fresh RPMI (5% FCS, no geneticin) were added to each well. Cells were cultured for an additional 15 h. Culture medium was removed and cells were washed with modified Hank's Balanced Salt Solution (HBSS) (without NaCl, containing 0.5% BSA, 0.1% glucose, 10 mmol/l HEPES, 0.5 mmol/l isobutylmethylxanthine) with addition of 220 mmol/l sucrose. Patient, control and standard

plasma (10 μl) diluted with 90 μl modified HBSS were incubated in triplicate with CHO-JP26 and, in another 96-well plate, with CHO-JP0 cells for 2 h at 37°C . The hypotonic supernatant was removed, and stored at -20°C until assayed for cAMP. To test parallelism of the assay, the effect of dilution of DS plasma on cAMP production was studied.

Biochemical analyses

The following assays were used: for T_4 , in-house RIA; FT_4 , RIA (Delfia, Pharmacia, Woerden, The Netherlands); TSH, ultrasensitive fluoroimmunoassay (Pharmacia, Woerden, The Netherlands); TSH binding inhibitory immunoglobulin (TBII) and thyroid peroxidase antibodies (TPO-Ab) (Brahms Diagnostica, Berlin, Germany); cAMP, RIA (^{125}I assay kit, Immunotech, Marseilles, France).

Statistical analysis

Results are given as means $\pm$ s.d. and ranges. Bioassay results are expressed as extracellular cAMP concentrations (pmol/well) obtained from incubation with CHO-JP26 cells of which cAMP levels from incubation with CHO-JP0 cells were subtracted. Plasma immunological TSH concentrations of patients and controls were substituted into the standard (IRP-TSH in pooled human plasma) regression equation to obtain patient-TSH equivalent cAMP response. Data were compared by two-tailed Student's *t*-test. Significance levels were set at $P < 0.05$.

Results

The bioassay shows a dose-response curve that is linear from baseline value TSH = 1.8 mU/l (pooled plasma) to at least TSH = 40 mU/l (pooled plasma spiked with IRP-TSH). The slope of the line (reflecting the sensitivity of the assay) was 0.67 ± 0.14 (range 0.47–0.86) and the intercept 15.0 ± 5.5 pmol cAMP/well (range 6.9–24.8) derived from 12 bioassays. The overall intra-assay coefficient of variation for the bioassay was less than 15%. The cAMP response in CHO-JP0 cells was always equal to the response observed by stimulation of pooled plasma and did not increase with the addition of IRP-TSH (data not shown). Since the available plasma sample sizes of most patients and controls were very small, we had to omit the preparation of multiple dilutions of these samples to evaluate parallelism with the standard curve. The effect of dilution of plasma of one DS patient on cAMP production in CHO-JP26 cells was determined. The TSH concentration in plasma was 17.9 mU/l. The use of 10, 7.5, 5.0 and 2.5 μl plasma in the bioassay resulted in the generation of 16.5, 12.4, 7.5 and 5.3 pmol/cAMP per well respectively. The

Figure 1 Plasma TSH bioactivity measured in CHO cells transfected with the human TSH receptor. cAMP generated per well after the addition of (10% v/v) plasma of children with Down's syndrome and subclinical hypothyroidism (■) of children with IDDM (▲) or of standard plasma spiked with WHO International Reference Preparation of human TSH (○). The standard regression line is described by $\text{cAMP (pmol/well)} = 0.71 [\text{plasma TSH (mU/l)}] \pm 11.5$. Each point represents the mean of a triplicate determination. Shown is one of two representative experiments.

regression line constructed from these data has a slope of 0.86 with a Pearson correlation coefficient of 0.98.

FT₄ levels in DS patients and controls range from 14.3 to 18.5 pmol/l and 12.7 to 16.4 pmol/l respectively, and are all within the reference range (10–23 pmol/l). The mean plasma TSH concentration in DS children with subclinical hypothyroidism (6.5 ± 1.3 mU/l, range 4.8–8.9 mU/l) is significantly different from the mean plasma concentration in controls (1.3 ± 0.4 mU/l, range 0.6–2.0 mU/l) (plasma TSH reference range 0.4–4.0 mU/l).

The cAMP response generated by DS and control plasma in CHO-JP0 cells ranged from 4.7 to 6.0 pmol/well and was subtracted from the cAMP response obtained with CHO-JP26 cells. Mean cAMP responses generated by DS plasma (18.4 ± 3.9 pmol/well, range 13.1–24.8) and control plasma (14.3 ± 1.3 pmol/well, range 12.6–17.1) were not significantly different from TSH equivalent cAMP response calculated from the standard regression line: 16.3 ± 0.9 pmol/well and 12.5 ± 0.4 pmol/well respectively (Fig. 1).

Discussion

This is the first study in which plasma TSH bioactivity of DS children with subclinical hypothyroidism and no

signs of thyroid autoimmunity has been examined. The normal concentration of cAMP generated in CHO cells (stable transfected with the human TSH receptor) by patient's plasma points to a normal TSH bioactivity.

The CHO bioassay is mainly used to detect plasma thyroid-stimulating antibodies (12, 13). Investigation of TSH bioactivity is an option when a discrepancy between clinical or biochemical data and the plasma immunochemical TSH value is observed, probably due to variable carbohydrate structures of circulating TSH (14).

The cumbersome immunopurification and concentration of plasma TSH are widely used to exclude the action of unknown factors, activating or inhibiting cAMP response (7, 15). However, this procedure needs large quantities of blood (excluding the use of children's plasma) and has the main drawback that antibodies used in the immunopurification may recognize only particular glycosylation variants of TSH through which the intracellular signal transduction system may be activated to different degrees (16). We routinely pre-diluted 10 µl unfractionated plasma with 90 µl modified HBSS prior to incubation with CHO cells, as was suggested by Beck-Peccoz and Persani (7), through which artifacts in the TSH bioassay may be consistently diluted out.

Persani *et al.* (10) seeded 10 000 cells/well in 96-well plates and ran the assay after 48 h. We seeded 30 000–40 000 cells/well through which sensitivity and linearity of the assay increased in the low TSH region (data not shown). The shorter period (24 h) in the present assay between seeding the cells and running the test is of further advantage.

In summary, our data show that subclinical hypothyroidism encountered in DS children was not due to diminished TSH bioactivity. We hypothesize that the thyroid gland in these children needs an elevated 'TSH pressure' to produce sufficient thyroid hormones. Further studies are needed to explore the signal transduction pathways involved in thyroid hormoneogenesis in thyroid tissue of DS patients, in order to unravel the underlying cellular cause for subclinical primary hypothyroidism in these patients.

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