Estradiol decreases iodide uptake by rat thyroid follicular FRTL-5 cells

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Abstract

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Estradiol has well-known indirect effects on the thyroid. A direct effect of estradiol on thyroid follicular cells, increasing cell growth and reducing the expression of the sodium-iodide symporter gene, has been recently reported. The aim of the present investigation was to study the effect of estradiol on iodide uptake by thyroid follicular cells, using FRTL-5 cells as a model. Estradiol decreased basal iodide uptake by FRTL-5 cells from control levels of 2.490 ± 0.370 to 2.085 ± 0.364 pmol I$^-$/µg DNA at 1 ng/ml (P<0.02), to 1.970 ± 0.302 pmol I$^-$/µg DNA at 10 ng/ml (P<0.003), and to 2.038 ± 0.389 pmol I$^-$/µg DNA at 100 ng/ml (P<0.02). In addition, 4 ng/ml estradiol decreased iodide uptake induced by 0.02 mIU/ml thyrotropin from 8.678 ± 0.408 to 7.312 ± 0.506 pmol I$^-$/µg DNA (P<0.02). A decrease in iodide uptake by thyroid cells caused by estradiol has not been described previously and may have a role in goiter pathogenesis.

Introduction

Estradiol has a well-known indirect effect on thyroid function, increasing thyroxine-binding globulin, probably due to decreased clearance rather than increased production of thyroxine-binding globulin (1). This effect may be responsible for the variations in thyroid size during the menstrual cycle, as measured by ultrasonography (2).

A direct effect of estradiol increasing thyroid follicular cell growth and reducing the expression of the sodium-iodide symporter was recently described by one of us (3) but the net result of these actions on iodide uptake by thyroid cells is not known.

The objective of the present investigation was to study the effect of estradiol on iodide uptake by thyroid follicular cells under basal conditions and after stimulation with thyrotropin (TSH), using FRTL-5 cells as a model (4).

Material and Methods

Cell culture

FRTL-5 cells (American Type Culture Collection, Rockville, MD, USA) were maintained in Ham's F12 Coon's modified medium supplemented with 5% calf serum (Gibco

BRL-Life Technologies, Gaithersburg, MD, USA), 5 µg/ml transferrin, 10 µg/ml insulin, 1 mIU/ml TSH, 1 µg/ml amphotericin B (Bristol-Myers Squibb, Santo Amaro, SP, Brazil) and 20 µg/ml amikacin (Bristol-Myers Squibb), in a humidified incubator at 37°C/ 5% CO_2, with a change of medium every 3 to 4 days. All media contained phenol red.

Iodide uptake

FRTL-5 cells (5 x 10^5 per well) were plated onto 24-well plates. After 4 days, cells were washed twice with warm PBS and incubated in Ham's F12 Coon's modified medium supplemented with 5% calf serum, 5 µg/ml transferrin, 10 µg/ml insulin, 1 µg/ ml amphotericin B, and 20 µg/ml amikacin (2H medium). After starvation for 7 days (-TSH), cells were treated as follows, in 2 different experiments:

Estradiol effect on basal iodide uptake. Control group (2H medium), group treated with 1 ng/ml estradiol (2H + 1 ng/ml estradiol), group treated with 10 ng/ml estradiol (2H + 10 ng/ml estradiol), and group treated with 100 ng/ml estradiol (2H + 100 ng/ml estradiol). Twelve wells were used per treatment group. Iodide uptake was determined after 24 h.

Estradiol effect on iodide uptake stimulated with TSH. Control group (2H medium), TSH group (2H medium + 0.02 mIU/ml TSH), and TSH + estradiol group (2H + 0.02 mIU/ml TSH + 4 ng/ml estradiol). Six wells were used per treatment group. The treatment medium was changed once and iodide uptake was determined after 48 h.

Ethanol, used as solvent for estradiol, was added as necessary so all treatment media had a 0.1% final content.

Iodide uptake was measured 48 h after incubation with TSH and 24 h after incubation with estradiol, as described previously (5). Briefly, cells were washed twice with Hanks' balanced salt solution-HEPES (HBSS-H), pH 7.4, and incubated for 30 min at 37°C

with 0.5 ml of warm HBSS-H-0.4% albumin-10 µM sodium iodide-^{125}I (NEN Dupont Nez-033, Boston, MA, USA), approximately 0.1 µCi. After 30 min, well content was quickly aspirated, cells were washed twice with ice-cold PBS and 1 ml of 99% ethanol was added to each well. Plates were kept for 1-2 h at room temperature and the content of the wells was transferred to tubes. Radioactivity was counted for 5 min (Autogama-Cobra, Packard, Downers Grove, IL, USA) in 3 tubes containing 0.5 ml of 99% ethanol and 0.5 ml of ^{125}I solution. Iodide uptake is reported as picomole iodide per µg DNA per well (pmol I$^-$/µg DNA), with the total count representing 5,000 pM of iodide. Iodide uptake was calculated using the formula: (counts observed/total count) x 5,000 pmol I$^-$/µg DNA.

DNA was measured by the diphenylamine method (6). After ethanol removal, cells were washed twice with cold PBS, and cold 5% TCA was added for a period of 15 min to several days at 4°C. TCA was aspirated and a "developing solution" [20 ml A solution (500 mg diphenylamine + 45 ml glacial acetic acid + 1 ml 18 M H_2SO_4) + 8 ml ddH$_2$O + 280 µl 1% acetaldehyde] was added for 24 h. The results were read at 580 nm with a spectrophotometer (Incibrás, São Paulo, SP, Brazil) and calculated according to the standards.

All experiments were repeated at least twice and gave essentially the same results. The data shown are those of a representative experiment.

All reagents, unless stated, were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Statistical analysis

Nonparametric one-way analysis of variance (Kruskal-Wallis test) was used for comparison between multiple groups and the Mann-Whitney test was used to compare two groups. All P values were two-sided and

a P value of less than 0.05 was considered to indicate statistical significance.

Results

Effect of estradiol on iodide uptake in the absence of TSH

As shown in Figure 1, estradiol decreased iodide uptake at all concentrations tested compared to control (from 2.490 ± 0.370 to 2.085 ± 0.364 pmol I⁻/μg DNA at 1 ng/ml (P<0.02), to 1.970 ± 0.302 pmol I⁻/μg DNA at 10 ng/ml (P<0.003), and to 2.038 ± 0.389 pmol I⁻/μg DNA at 100 ng/ml (P<0.02)).

Effect of estradiol in combination with TSH on iodide uptake

As shown in Figure 2, 0.02 mIU/ml TSH increased iodide uptake from 1.492 ± 0.148 to 8.678 ± 0.408 pmol I⁻/μg DNA (P<0.006), and the addition of 4 ng/ml estradiol reduced the effect of TSH to 7.312 ± 0.506 pmol I⁻/μg DNA (P<0.02).

Discussion

In the present study we examined the effect of estradiol on iodide uptake by FRTL-5 cells. A direct effect of estradiol on thyroid follicular cells was expected because estrogen receptors are present in abundant amounts in these cells (7-9).

We were able to demonstrate a decrease in iodide uptake by estradiol-treated FRTL-5 cells in the presence and absence of TSH. This effect was expected on the basis of the estradiol-induced decrease in sodium-iodide symporter gene expression observed previously in the presence of TSH, but not when estradiol alone was used (3). Nevertheless, estradiol alone was able to block iodide uptake. This apparent discrepancy may have been due to a better sensitivity of the iodide uptake technique in measuring the effect of estradiol under these conditions when com-
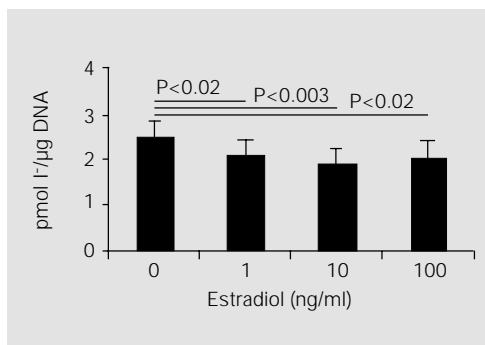

Figure 1 - Estradiol decreases basal iodide uptake in FRTL-5 cells. Cells (5 x 10⁵ per well) were grown for 4 days in 24-well plates, deprived of TSH for 7 days, and treated for 24 h with no estradiol, 1 ng/ml estradiol, 10 ng/ml estradiol or 100 ng/ml estradiol (12 wells per group). Results are reported as mean ± SD. Significance levels are shown at the top of bars (Mann-Whitney test). TSH, Thyrotropin.
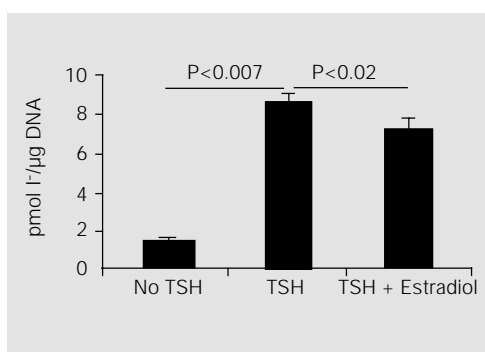

Figure 2 - Estradiol decreases TSH-stimulated iodide uptake in FRTL-5 cells. Cells (5 x 10⁵ per well) were grown for 4 days in 24-well plates, deprived of TSH for 7 days, and treated for 48 h with no TSH, 0.02 mIU/ml TSH or 0.02 mIU/ml TSH associated with 4 ng/ml estradiol, with one change (6 wells per group). Results are reported as mean ± SD. Significance levels are shown at the top of bars (Mann-Whitney test).

pared to the semi-quantitative method used to measure the very low sodium-iodide symporter gene expression in the absence of TSH.

This report is the first to demonstrate a decrease in iodide uptake by FRTL-5 induced by estradiol. The observed effect was quite mild and could have been influenced by the presence of estrogenic activity in the medium (steroid activity of phenol red present in the medium, or inhibitory steroids in serum). In addition, the concentrations of estradiol used were supra-physiological and experiments to study estrogen associated with an antagonist of its action were not performed, so that some nonspecific effect cannot be excluded. Nevertheless, since the decrease of the sodium-iodide symporter gene expression, described previously using similar experimental conditions, was fully reversed by ICI182780 (3), an estrogen antagonist, we would expect the same to occur for iodide uptake.

The mechanisms mediating the function

of thyroid follicular cells are complex (10,11), and how estradiol affects iodide uptake by FRTL-5 cells is not understood. Estrogen may have a direct nuclear effect on sodium-iodide symporter gene expression, associated or not with a decrease in the ability of the sodium-iodide symporter to carry iodide. Another possible explanation is the production of some estradiol-induced growth factor able to decrease iodide uptake. Some growth factors have shown this ability, like activin A (12), epidermal growth factor (13,14), TGF-α (15) and basic fibroblast growth factor (16). Thyroid follicular cells have been shown to synthesize growth factors like IGFII (17), TGF-ß (18,19) and endothelin-1 (20), and have been observed in human thyroid (21), so that estrogen-induced growth factor production could potentially help to explain the effect of estradiol.

No study has been conducted thus far to verify if the estradiol-induced iodide uptake by follicular cells is accompanied by a decrease in cell iodide content. It is well known that the decrease in iodide content of thyroid cells makes these cells more susceptible to the effects of TSH (22,23). If there is a decrease in the iodide content of thyroid follicular cells induced by estradiol, this may help to explain the increased prevalence of goiter in women (24-26), even when their TSH levels do not differ from those observed in men. More studies should be done to better understand the functional interrelation of estradiol and thyroid follicular cells.

Acknowledgments

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