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Beta-adrenoceptor antagonist therapy in hyperthyroidism: effects on lymphocyte metabolic activity and the relation between aerobic and anaerobic metabolism in lymphocytes ☆

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Abstract

Thyroid hormones are of major importance for the regulation of metabolic activity at the cell level in humans. An increased heat production has been demonstrated in lymphocytes from patients with hyperthyroidism. However, the lymphocyte oxygen consumption was not increased, indicating an increased dependency on anaerobic metabolism in lymphocytes exposed to thyroid hormone excess.

The present study focused on the effects of β-adrenoceptor antagonists on the metabolic activity in lymphocytes from hyperthyroid patients. Lymphocytes from patients on treatment with these drugs showed a heat production that was at a level similar to that of cells from normal subjects and significantly lower than lymphocytes from patients before treatment for hyperthyroidism and not on β-blockers. Similarly, lymphocyte oxygen consumption was of the same order in hyperthyroid patients on β-blockers as in euthyroid subjects.

Treatment with β-adrenoceptor antagonists seems to preclude an increase in the metabolic activity of lymphocytes, otherwise present in hyperthyroid patients. These data suggest that β-adrenoceptor activity may play a role in the enhanced metabolic activity of these cells during thyroid hormone excess. A stimulating effect of thyroid hormones on the sensitivity of adrenoceptors might thereby be of greater importance than the calorigenic effect exerted

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by thyroid hormones via nuclear binding. The metabolic response to thyroid hormones may be specific in lymphocytes and different between tissues.

Keywords: Adrenergic receptor; Calorimetry; Hyperthyroidism; Lymphocyte; Metabolism; Thyroid hormone

1. Introduction

Thyroid hormones play an important role in the regulation of metabolic activity in the body, reflected by increased basal metabolic rate (BMR) in hyperthyroidism and decreased BMR in hypothyroidism. In accordance with this, hyperthyroid patients suffer from, for instance, increased sweating, hypersensitivity to heat, weight loss and increased heart rate. The precise mechanisms for the calorigenic effect of thyroid hormones remain uncertain. An effect on the activity of cell membrane bound Na/K ATP-ase has been proposed [1] but later challenged [2].

Microcalorimetry has been used for studies which showed the increased metabolic activity in erythrocytes [3] and lymphocytes [4] from hyperthyroid patients. This effect on erythrocyte and lymphocyte cell metabolism however could not be explained specifically by an increased Na/K pump activity, because the contribution of ouabain-inhibited thermogenesis was of the same relative order in the hyperthyroid state as in the euthyroid state achieved after treatment [3,5]. Further evaluation of the increased metabolic activity in lymphocytes from hyperthyroid patients focused on the roles of aerobic and anaerobic metabolism. It has been demonstrated recently that an increased activity along anaerobic metabolic pathways has a major role in the increased heat production in these cells in the hyperthyroid state [6].

The therapeutic approach towards patients with hyperthyroidism often includes the use of β-adrenoceptor antagonists, drugs that have been found to be useful adjunctive tools to relieve symptoms but without specific effects on thyroid hormone production or on the effects of thyroid hormones on cell function [7]. The present study was conducted in order to study to what extent treatment with β-adrenoceptor antagonists affects the metabolic activity of lymphocytes in hyperthyroidism. In addition, it aimed at further evaluation of the role of anaerobic processes in these cells during the hyperthyroid state and whether it is the level of metabolic activity or the increased thyroid hormone concentration that per se is responsible for the transfer towards an increased role of anaerobic processes in lymphocytes from hyperthyroid patients.

2. Material and methods

2.1. Patients and controls

Eight patients, 6 females and 2 males aged 36–63 years, with diffuse toxic goitre, clinical signs of hyperthyroidism and increased serum levels of free throxine (FT4),

mean ± SE = 93.4 ± 11.8 pmol l^{-1}, and of free triiodo-thyronine (FT3), 27.9 ± 2.4 pmol l^{-1}, were studied when they were on β-adrenoreceptor antagonist (β-blockers) therapy but before they received specific antithyroid treatment. The drugs used were propranolol or metoprolol in regular doses.

Another group of eleven patients, ten women and one man aged 34–89 years, with hyperthyroidism and serum levels of FT4 at 69.2 ± 5.5 pmol l^{-1} and of FT3 at 24.9 ± 3.2 pmol l^{-1} (not significantly different from the FT4 and FT3 levels in the group above of patients on β-blockers) had been studied [6] before and after treatment leading to euthyroidism when FT4 was 21.1 ± 2.2 pmol l^{-1} and FT3 at 6.8 ± 0.6 pmol l^{-1}.

The data from the group on β-blockers were compared with data from these patients before and after treatment and also with calorimetric data from a group of healthy control subjects, 9 women and 9 men, without signs of thyroid disease, aged 24–49 years. The heat production by lymphocytes in all the healthy subjects was studied together with oxygen consumption by the cells of 10 of them.

2.2. Lymphocyte preparation

Suspensions of lymphocytes were prepared within 3 h from a fresh venous blood sample. The lymphocytes were separated by gradient centrifugation using isopaque Ficol. Phagocytic cells were eliminated magnetically after phagocytosis of iron powder [8]. One sample was then directly prepared for heat production measurements by resuspension of lymphocytes in cell-free autologous plasma at a concentration of (1–1.5) × 10^9 lymphocytes l^{-1}. The calorimetric measurements were then carried out immediately [8].

2.3. Calorimetric measurements

The lymphocyte suspension was kept in a 1.2-ml steel ampoule during the measurement, performed by a microcalorimeter of heat conduction type at steady state and at 37°C as described earlier [8]. The cell concentration was chosen to avoid crowding of the lymphocytes so that measurements could be performed without stirring. Data on heat production rate thus obtained were expressed in pW per cell (pJ s^{-1} per cell).

2.4. Measurement of oxygen consumption

The contribution of aerobic metabolism to lymphocyte energy expenditure was studied in separate experiments performed simultaneously with the calorimetric measurement. The oxygen consumption in a plasma suspension of lymphocytes, prepared as for the calorimetric measurement, was continuously monitored by a Clark oxygen electrode; volume 1 ml at 37°C. (Hansatech, Surrey, UK). The oxygen-dependent heat energy production was then calculated from the product of the rate of oxygen consumption (mol s^{-1} per cell) and the enthalpy change for glucose, $\Delta H = -2931$ kJ per mol glucose. ΔH was calculated from thermodynamic

data [9]. A continuous metabolic activity in vitro for more than 5 h has previously been demonstrated in lymphocytes when glucose was present as the only substrate [10,11].

2.5. Thyroid hormones

The thyroid hormone concentrations were determined by routine radioimmunoassays in blood samples drawn at the same time as those for lymphocyte preparations.

2.6. Statistics

Comparison between means was made by the Student's t-test. The coefficient of correlation between variables was calculated according to the method of least squares.

3. Results

In the hyperthyroid patients not treated with β blockers, the heat production value (P) was 3.37 ± 0.25 (SE) pW per cell before ($n = 11$) and 2.50 ± 0.11 pW per cell after ($n = 10$) treatment for hyperthyroidism ($p < 0.01$) as compared to 2.32 ± 0.109 pW per cell in a control group ($n = 18$), Fig. 1. The value for heat

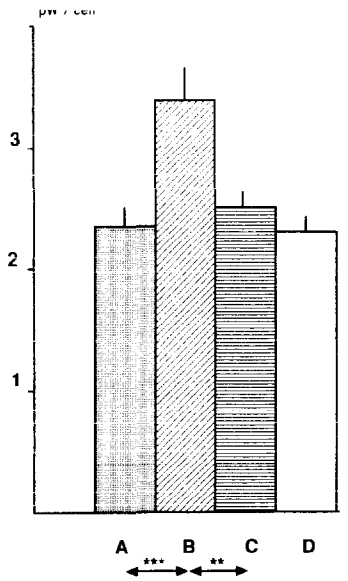

Fig. 1. Total heat production rate in lymphocytes from: A, hyperthyroid patients on β-blockers but before specific antithyroid therapy ($n = 8$); B, hyperthyroid patients not on β-blockers and before specific antithyroid therapy ($n = 11$); C, the same patients as in group B but after specific antithyroid therapy; D, healthy subjects ($n = 18$). ** $= p < 0.01$; *** $= p < 0.005$. Vertical bars indicate SE.

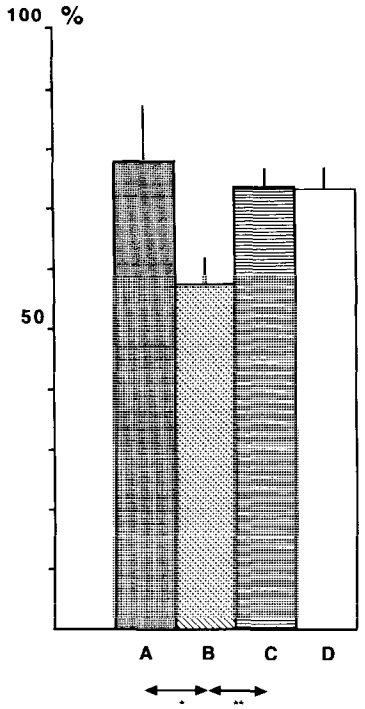

Fig. 2. The relative contribution of aerobic metabolism to the total heat production rate in lymphocytes from: A; hyperthyroid patients on β-blockers but before specific antithyroid therapy ($n = 8$); B, hyperthyroid patients not on β-blockers and before specific antithyroid therapy ($n = 11$); C, the same patients as in group B but after specific antithyroid therapy; D, healthy subjects ($n = 18$). * $= p < 0.05$, ** $= p < 0.01$. Vertical bars indicate SE.

production that was calculated from oxygen consumption rate is termed the aerobic contribution (O_2-P) to the heat production (P) of the lymphocytes. O_2-P thus obtained amounted to 1.83 ± 0.11 pW per cell in the hyperthyroid patient group before and 1.83 ± 0.08 pW per cell after treatment. In the 10 control subjects studied this way, O_2-P was 1.71 ± 0.16 pW per cell.

The relative contribution of O_2-P to P corresponded to $56.8 \pm 4.4\%$ before and to $73.7 \pm 3.2\%$ after treatment for hyperthyroidism ($p < 0.01$) as compared to $73.4 \pm 4.4\%$ in the 10 euthyroid controls, Fig. 2.

In the hyperthyroid patients receiving β-blockers ($n = 8$), P was 2.34 ± 0.16 pW per cell, which was significantly lower than the P value registered in the group of untreated hyperthyroid patients without β-blockers ($p < 0.005$), and not different from controls, Fig. 1. The value for O_2-P in the group of hyperthyroid patients on β-blockers was 1.87 ± 0.28 pW per cell, corresponding to $78.2 \pm 8.6\%$ of P, not different from the above post-treatment values or from controls, Fig. 2.

4. Discussion

β-Adrenoceptor antagonists are often used in clinicial practice to alleviate symptoms in patients with hyperthyroidism but in general are considered not to interfere with the effects of thyroid hormones on cell function. One of the main findings in the present study was, however, that heat production in lymphocytes from hyperthyroid patients treated with β-adrenoceptor antagonists, was no different to the controls or to lymphocytes from patients already treated for hyperthyroidism. Thus, treatment with β-adrenoceptor antagonists seems to moderate metabolic activity in the lymphocytes so that the increased calorigenic activity otherwise seen in these cells from hyperthyroid subjects was precluded. Obviously, this difference could not be explained by different thyroid hormone levels. In fact the free thyroxine level was even slightly higher in the group on β-blockers. A decreased heat production rate has also recently been found in skeletal muscle exposed to the β-adrenoceptor antagonist propranolol [12].

A more likely explanation for the persistently normal metabolic activity of lymphocytes from hyperthyroid patients on β-blockers would be a direct interference of the drug on lymphocyte metabolism through specific adrenergic receptors. In recent years several investigators have made use of lymphocytes for studies of the importance of adrenergic receptors for immunological activity, demonstrating the presence of adrenergic receptors on these cells [13,14]. It is well known that catecholamines exert various metabolic effects. Although circulating levels of catecholamines may remain normal in hyperthyroidism [7], thyroid hormone excess has been thought to increase the sensitivity to catecholamines and would thereby be able to contribute to the development of symptoms of adrenergic type in these patients. This explanation has not however gained general acceptance [7] and differences may be present between organs with regard to interaction between thyroid hormones and adrenergic receptors [15]. A close relationship between the serum level of triiodothyronine and the β-adreno-receptor density on lymphocytes, however, has been reported, a finding that supports a stimulatory influence of thyroid hormones on lymphocyte adrenergic receptors [16].

In the present study, β-adrenoceptor antagonist therapy was associated with thermogenesis at a normal level in the lymphocytes from hyperthyroid patients. This could even mean that an increased sensitivity to the effect of catecholamines on lymphocyte thermogenesis is of major importance for the calorigenic effects of thyroid hormone excess in these cells. This would be in accord with the general idea that treatment with these drugs does not interfere with the effect of thyroid hormones on cell function. However, this would also mean that the specific influence of thyroid hormone excess on lymphocyte thermogenesis is relatively modest. Lymphocytes are known to carry nuclear receptors for thyroid hormones [17]. Possibly, the importance of nuclear binding of triiodothyronine for calorigenic activity in lymphocytes may be inferior to that exerted through adrenergic receptors.

According to earlier studies [11,18], lymphocytes possess enzymatic systems for aerobic and anaerobic glucose metabolism. During basal conditions, up to 75% of

the glucose utilized by lymphocytes has been estimated to be metabolized along aerobic pathways, while the role of glycolysis increases during anaerobic conditions [11]. Although total body oxygen consumption is increased in the hyperthyroid state this does not seem to be the case in lymphocytes [5,6]. The effects of thyroid hormone excess on oxygen consumption may thus differ between tissues or types of cells. The relative shortage of oxygen in lymphocytes from hyperthyroid patients might then contribute to a shift towards anaerobic glucose utilization, necessary to supply energy-rich substances to meet the demands induced via increased adrenergic receptor sensitivity in the hyperthyroid state.

In conclusion, the present data demonstrate increased metabolic activity in lymphocytes from hyperthyroid patients, a phenomenon that seems to be precluded by the use of β-blockers. It is therefore suggested that the increased thermogenesis in lymphocytes from hyperthyroid patients might be mediated via adrenergic receptors, probably due to an increased sensitivity during thyroid hormone excess, rather than via direct effects on nuclear thyroid hormone receptors in these cells. Microcalorimetry seems to be suitable for further studies on the mechanisms of the calorigenic effects of thyroid hormones, which thus still seem complex.

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