Increased lymphocyte thermogenesis in hyperthyroid patients. Role of Na/K pump function. Evaluation of aerobic/anaerobic metabolism

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The role of the Na/K pump for the increased cell energy expenditure in hyperthyroidism was studied by measuring total lymphocyte heat production rate in samples with and without ouabain inhibition of Na/K ATP-ase. In addition, the relative contribution of aerobic processes to lymphocyte thermogenesis was calculated from oxygen consumption measurements. In 12 patients with clinical and laboratory hyperthyroidism total lymphocyte heat production rate was 3.19 ± 0.21 pW/cell, significantly higher than in 7 patients with subclinical hyperthyroidism (2.14 ± 0.11 pW/cell) and in 15 euthyroid subjects (2.26 ± 0.11 pW/cell) (p < 0.001). The relative decrease in lymphocyte heat production rate after ouabain, giving a quantitative measure of the activity of the Na/K ATP-ase and reflecting the importance of Na/K pump function for the overall rate of lymphocyte metabolism, was not significantly different between the groups: 19.5 ± 3.6% in hyperthyroid patients, 14.2 ± 2.3% in subclinical hyperthyroid patients and 17.8 ± 3.1% in euthyroid subjects. According to the rate of lymphocyte oxygen consumption, aerobic processes represented 58.4 ± 6.7% of total lymphocyte energy expenditure in hyperthyroid patients, not significantly different from subclinical hyperthyroidism (62.6 ± 8.4%) or from euthyroidism (66.6 ± 2.7%). These data do not support the hypothesis of a specific role of the Na/K pump function for the increased cell thermogenesis in hyperthyroidism and indicate a parallel stimulation of aerobic and anaerobic processes by thyroid hormone excess.

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Specific thyroid hormone stimulation of Na/K ATP-ase dependent Na/K pump function has previously been suggested to be of major importance for an increased cellular thermogenesis in hyperthyroidism (1). Using microcalorimetry (2), we have demonstrated an increased heat production rate in erythrocytes (3) and lymphocytes (4) in hyperthyroidism as well as a decreased heat production rate in platelets and adipocytes in hypothyroidism (5). To evaluate the role of the Na/K pump function for the increased cellular thermogenesis in hyperthyroidism, erythrocyte heat production rate has been measured with and without ouabain inhibition of Na/K ATP-ase. The relative contribution of the Na/K pump power to total heat production rate was found to be in the same order in the hyperthyroid as in the euthyroid state (3). These data did not support the hypothesis of a specific effect of thyroid hormones on Na/K pump function. However, since nuclear receptors are regarded to be of major importance for thyroid hormone effects at the cell level (6), data from erythrocyte studies cannot be extrapolated to other cells. In order further to evaluate the role of Na/K pump function for the increased thermogenesis in nucleated cells we extended our previous lymphocyte investigations by studying heat production rates with and without Na/K ATP-ase inhibition by ouabain in lymphocytes from hyperthyroid patients. In addition, lymphocyte heat energy production was specifically calculated from the oxygen consumption rate in vitro in order to estimate the relative importance of oxygen-dependent metabolism for lymphocyte thermogenesis.

Subjects and methods

Patients

Group A: 12 patients with clinical and laboratory signs of hyperthyroidism due to diffuse goitre (9F and 3M, aged 29–65 years). All had suppressed thyrotropin (TSH) levels. Their mean (±SEM) level of free thyroxine (FT4) was 70.5 ± 20.4 pmol/l (reference range 12–25 pmol/l) and of free triiodo-thyronine (FT3) 24.0 ± 2.6 pmol/l (reference range 3.3–8.2 pmol/l).

Group B: 7 female patients without evident clinical signs of hyperthyroidism, 26–59 years old. One patient had an autonomous adenoma while the others had diffuse or multinodular goitre. All of them had suppressed TSH levels. The mean serum FT4 level was
slightly increased (29.7 ± 2.0 pmol/l), while the mean serum FT3 level was within the reference range. 6.8 ± 0.6 pmol/l (subclinical hyperthyroidism).

Controls
Fifteen healthy subjects without goitre (8F and 7M). All were studied with regard to total lymphocyte heat production rate. The Na/K pump power in lymphocytes was determined after ouabain inhibition in nine of them. In six of the subjects the aerobic lymphocyte energy expenditure was estimated from the lymphocyte oxygen consumption, which was measured separately in vitro. None of the patients or controls were on beta blockers at the time of blood sampling. All had given informed consent to blood sampling.

Lymphocyte preparation
Suspensions of lymphocytes were prepared from a 30 ml venous blood sample. The lymphocytes were separated by gradient centrifugation in Isopaque Ficol. Phagocytic cells, granulocytes and monocytes, were eliminated by a magnetic technique after phagocytosis of iron powder. The separation process was completed within 3 h. For total heat production measurements a sample was directly prepared by resuspension of lymphocytes in cell-free autologous plasma at a concentration of 1–1.5 × 10⁶ lymphocytes/l.

For Na/K pump studies, two samples of the lymphocyte suspension were prepared in saline. Each sample contained 175 µl. Ouabain (25 µl of 15 mmol/l ouabain octahydrate. Strophantin G) was added to one sample and 25 µl of 9 g/l saline was added to the other sample. Both suspension samples were then incubated at 37°C for 1 h before the addition of 0.8 ml of cell-free autologous plasma to each sample. The final lymphocyte concentration was 1–1.5 × 10⁹ cell/l. The final ouabain concentration in the cell suspensions was 3.75 × 10⁻⁴ mmol/l, a concentration previously found to give the maximum inhibition of the Na/K pump function.

The calorimetric measurements were carried out immediately after the preparation (7).

Calorimetric measurements
The lymphocyte suspension was enclosed in a 1.2 ml steel ampoule during measurement of heat production performed by a microcalorimeter of heat conduction type (LKB 10700) at steady state and at 37°C as described earlier (7). Data on heat production rate thus obtained were expressed in pW/cell (pJ·sec⁻¹·cell⁻¹).

Determination of Na/K pump power
The energy expended through Na/K pump activity is dependent on the activity of cell membrane bound Na/K ATP-ase, which can be inhibited by ouabain. In separate experiments heat production was measured simultaneously in the two samples prepared with or without ouabain. The difference in heat production rates between the two samples gives a quantitative measure of the activity of the Na/K ATP-ase, reflecting the role of the Na/K pump activity for the overall rate of lymphocyte metabolism.

Measurement of lymphocyte oxygen consumption
The contribution of aerobic metabolism to total lymphocyte energy expenditure was studied in separate experiments where the rate of oxygen consumption in a plasma suspension of lymphocytes was continuously monitored using a Clark oxygen electrode; volume 1 ml at 37°C (Hansatech, Surrey, UK). The oxygen-dependent heat energy production was calculated from the product of the rate of oxygen consumption (mol·sec⁻¹·cell⁻¹) and the enthalpy change, ΔH = – 2931 kJ·(mol glucose)⁻¹. ΔH, the thermodynamic quantity for the combustion of glucose to carbon dioxide and water, was calculated from thermodynamic data (8).

Thyroid hormones
The thyroid hormone concentrations were measured in blood samples drawn at the same time as those for the lymphocyte preparations. TSH was determined by a sensitive radioimmunoassay from Amersham UK, FT4 and FT3 by radioimmunoassays from Wallac, Finland.

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

Results
Total lymphocyte heat production rate
The hyperthyroid patients in group A had a mean (± SEM) lymphocyte heat production rate of 3.19 ± 0.21 pW/cell, significantly higher than the controls (2.26 ± 0.11 pW/cell) (p < 0.001). In group B (subclinical hyperthyroidism) the corresponding value was 2.14 ± 0.11 pW/cell (non-significant (NS) vs controls). When calculated for groups A + B a significant correlation appeared between total lymphocyte heat production rate on the one hand and the serum FT4 values (r = 0.49, p < 0.05) and heart rate (r = 0.57, p < 0.05) on the other.

Na/K pump energy expenditure
Total lymphocyte heat production rate was significantly lower in samples with ouabain for all three groups
studied (Fig. 1). The difference between the values obtained in the samples without or with ouabain, reflecting Na/K pump power, constituted 19.5 ± 3.6% of the value without ouabain in the hyperthyroid patients in group A, which was not statistically different from the corresponding values in patients with subclinical hyperthyroidism (group B) (14.2 ± 2.3%), or in the euthyroid controls (group C) (17.8 ± 3.1%).

**Oxygen-dependent energy expenditure**

These data were calculated for eight of the patients in group A, for five patients in group B and for six of the controls (Fig. 2). The mean values obtained were 1.89 ± 0.14 pW/cell in the hyperthyroid patients (group A), 1.36 ± 0.20 pW/cell in patients with subclinical hyperthyroidism (group B) and 1.50 ± 0.12 pW/cell in the controls (NS vs group A). Out of the total lymphocyte heat production rate found in the same patients, these figures, reflecting aerobic metabolic pathways, represented 58.4 ± 6.7% in group A and 62.6 ± 8.4% in group B, not significantly different from the corresponding value in group C (66.6 ± 2.7%).

**Discussion**

A major problem in studies of the role of Na/K pump activity for cell energy expenditure is the technique whereby this function can be reliably measured. Earlier studies made use of changes in oxygen consumption rate after inhibition of Na/K ATP-ase by ouabain (1). The drawback of this method is that it estimates energy consumption indirectly and thereby only aerobic processes. More recent studies have made use of determinations of the number of Na/K pump sites by measuring ouabain binding sites. An increased number of ouabain
binding sites has thus been found in skeletal muscle (9) and leucocytes (10) from hyperthyroid subjects, while decreased sodium pump activity (11). Na/K ATP-ase activity (12, 13) and number of sodium pump sites (14) have been found in erythrocytes in hyperthyroidism. However, the relationship between the number of ouabain binding sites and the energy expenditure by Na/K ATP-ase dependent sodium transport has not been clarified.

Lymphocytes are easily accessible nucleated cells, previously demonstrated to carry thyroid hormone receptors (15, 16), and are therefore useful for microcalorimetric studies of thyroid hormone effects and thereby more representative for other tissue cells than erythrocytes. The finding of an increased lymphocyte heat production rate in hyperthyroidism reflects directly the net calorigenic effect of thyroid hormones on lymphocytes and this is supported by the significant correlations between the lymphocyte heat production rate on the one hand and the free thyroxine level and heart rate on the other in the present study.

However, the difference in lymphocyte heat production rate between samples with and without ouabain, corresponding to Na/K pump power, did not specifically account for the increased total lymphocyte heat production rate. Thus, although the absolute value (pW/cell) of the Na/K pump power was higher in hyper- compared to euthyroidism, the relative contribution of this function to total lymphocyte thermogenesis was of the same order in hyperthyroidism, clinical as well as subclinical, and in euthyroid controls. In an earlier study on erythrocyte thermogenesis, we found that similarly obtained data on Na/K pump power were well correlated to the change in the intracellular sodium level (3). It is therefore justified to assume that the present findings on lymphocyte Na/K pump power reflect the functional activity of the Na/K pump in these cells.

The present data on lymphocyte metabolism are in accordance with an earlier report that the ouabain binding capacity in lymphocyte membranes did not increase in parallel with the thyroid hormone level in hyperthyroidism (17). Furthermore, our present data agree with earlier results from mammalian skeletal muscle (18) and rat hepatocytes (19), where Na/K transport and Na/K ATP-ase thermogenesis respectively, could not be demonstrated to be of major importance for the increased thermogenesis in the hyperthyroid state.

The mechanism whereby thyroid hormones regulate cell thermogenesis is thus still unclear. We have previously found that the increased heat production rate in erythrocytes from hyperthyroid patients is due to a parallel stimulation in aerobic and anaerobic pathways and that no less than about 60% of erythrocyte heat production rate takes place along the Embden Mayerhof pathway, corresponding to anaerobic metabolism (20). In the present study we found that the relative contribution from oxygen-dependent glucose combustion to total lymphocyte heat production rate was of the same order in both clinical and subclinical hyperthyroidism as in the euthyroid state, corresponding to about 60% of total energy expenditure in the lymphocytes. These data suggest that thyroid hormones stimulate aerobic as well as anaerobic metabolic pathways. Opposite to our previous findings in erythrocytes, the present study shows that aerobic pathways dominate in lymphocytes.

Indirectly, our findings indicate that no less than 40% of energy consumption in lymphocytes, taking place along anaerobic pathways, would have been missed if studies of thyroid hormone effects on lymphocyte metabolism had been based exclusively on oxygen consumption measurement. As a matter of fact, in the present study the value for lymphocyte heat production rate was not significantly higher for the hyperthyroid patients compared to healthy subjects when calculated from oxygen consumption, while there was a significant difference between the corresponding values obtained by direct microcalorimetry (Fig. 2).

In conclusion, by using direct microcalorimetry, we found that the relative contribution of the Na/K pump power to total lymphocyte heat production rate in hyperthyroidism was of the same order as in euthyroid controls, indicating that the energy expenditure by the Na/K pump function is not of specific importance for the increased lymphocyte thermogenesis demonstrated in hyperthyroidism. Furthermore, aerobic and anaerobic pathways in lymphocytes seem to be stimulated in parallel in hyperthyroidism, the aerobic processes contributing to about 60% of total energy expenditure in these cells.

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