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MICROCALORIMETRIC STUDIES OF HUMAN BLOOD CELLS IN THYROID DISEASE *

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SUMMARY

Using microcalorimetry, an increased heat production rate could be demonstrated in erythrocytes and lymphocytes from hyperthyroid patients. Hyperthyroidism was found to stimulate energy expenditure in erythrocytes along the anaerobic as well as along the aerobic pathway. The sodium-potassium pump does not account for the increased erythrocyte heat production rate in hyperthyroidism. In hypothyroidism, platelet heat production rate was found to be significantly decreased, returning to normal after treatment. In subclinical hyper- and hypothyroidism, lymphocyte energy expenditure was found to be normal.

These results show that microcalorimetric measurements on blood cells give useful information concerning the peripheral effect of thyroid hormones.

INTRODUCTION

The thyroid gland synthesizes hormones that are of major importance for regulation of metabolism of almost all cells of the human body. As a consequence, many physiological and biochemical functions are under the influence of this organ. However, despite an enormous amount of research in the last 30 years the mechanism of thyroid hormone action is still unclear.

The diagnosis of derangement of thyroid function is based on the clinical features in the particular patient and on serum concentration of thyroid hormones. There is not at present a parameter that reflects the consequences of changes in thyroid hormone levels with regard to cellular function. It is therefore sometimes difficult to make such an evaluation taking into account that the clinical features of the disease might differ considerably between different patients with the same degree of changes in thyroid hormone levels. Old patients, for example, show often less pronounced symptomatology compared to other age groups. Thus, the concentration of thyroid hormones does not necessarily indicate to what extent the tissues react to the hormonal influence in different patients. In clinical work the choice of therapy for each single patient would be easier if the state of metabolic derangement at the cell level had been known.

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Attempts have been made in the last 20 years to use microcalorimetry to provide an index of cell metabolism in thyroid dysfunction. Mainly human blood cells have been the object of these observations.

RESULTS AND DISCUSSION

Levin (ref 1) has been the first to report the results of flow calorimetric measurements of heat production in leucocytes, platelets and plasma from hyper- and hypothyroid patients. Increased leucocyte heat production rate was found in hyperthyroidism whereas decreased values were observed in hypothyroidism.

Monti and Wadsö (ref 2) found that heat production rate in erythrocytes from hyperthyroid subjects was elevated (102 ± 15 mW/l erythrocytes) and decreased (85 ± 14) significantly ($p < 0.05$) after treatment. The initial value was significantly ($p < 0.001$) higher than the corresponding value for normal subjects, 78 ± 5 mW/l, see table 1. These and later measurements were performed by the authors using a static calorimetric system. A good correlation was noted between calorimetric values and the clinical condition of the patients. The action of thyroid hormones at the cellular level is expected to be carried out by attachment of the hormones to the nuclear receptors in the target cells (ref 3). The results of the present work, however, show an increased metabolism in erythrocytes from hyperthyroid subjects although these cells do not carry a nucleus. These studies support the conclusion that thyroid hormones have the capability to stimulate cell metabolism also by other mechanisms than through nuclear receptors.

More detailed calorimetric studies were apparently needed to clarify which pathways are activated by the thyroid in the human erythrocytes. Previous studies (ref 4) have shown an increased oxygen consumption in erythrocytes from hyperthyroid subjects indicating an increased energy extraction via the hexose monophosphate shunt, nothing being known about the energy flow via the Embden-Meyerhof pathway in hyperthyroidism. Monti et al. (ref 5) have therefore performed further studies in the erythrocytes from hyperthyroid subjects, using sodium fluoride to inhibit enolase and thus stop substrate utilization through the anaerobic pathway. The decrease in heat production rate in samples with sodium fluoride corresponds to the anaerobic contribution, whereas the values from samples with sodium fluoride reflect aerobic processes. Before treatment, erythrocyte total heat production for 10 hyperthyroid subjects was 120 ± 2 mW/l, significantly higher ($p < 0.001$) than the corresponding value after treatment, 99 ± 2, as well as the value for 14 healthy subjects, 108 ± 2 mW/l. The sodium fluoride inhibitable rate was 73 before and 63 mW/l after treatment ($p < 0.01$), corresponding to 61 and 64 % of the total heat production rate in the hyperthyroid and euthyroid state respectively, not different from the percentage value for control subjects, 61 %. Heat production rate in the presence of sodium fluoride was 47 before and 36 mW/l after treatment ($p < 0.001$), representing 39 and 36 % of the total values, respectively, see table 1.

TABLE 1
Heat production rate in erythrocytes from hyperthyroid patients before and after treatment. The variation of value in the different studies is due to the fact that the calorimetric measurements were performed under different experimental conditions.

Monti and Wadsö 1976	Heat production, mW/l ± SD before treatment (10) 102±15 p<0.05 after treatment (8) 85±14	Controls (8) 78±5 p<0.001
Monti, Hedner, Ikomi-Kumm and Valdemarsson 1987	Total heat production, mW/l±SEM before treatment (10) 120±2 p<0.001 after treatment (10) 99±2 Glycolytic component before treatment (10) 61 % after treatment (10) 64 % Non-glycolytic component before treatment (10) 39 % after treatment (10) 36 %	Controls (14) 108±2 p<0.001 Controls (14) 61 % Controls (14) 39 %
Monti, Hedner, Ikomi-Kumm and Valdemarsson 1987	Total heat production, mW/l ±SEM before treatment (11) 131±4 p<0.01 after treatment (11) 112±4 Sodium potassium pump before treatment (11) 11.2±2 p>0.05 after treatment (11) 9±2	Controls (33) 107±13 p<0.001 Controls (33) 14.5 p>0.05

These data show that in erythrocytes from hyperthyroid patients, both the aerobic and the anaerobic pathways were stimulated, and that the relative contributions from the hexose-monophosphate (aerobic) and the Embden-Meyerhof (anaerobic) pathways to total cellular heat production are of the same magnitude in the hyperthyroid and euthyroid state. The results are in agreement with previous works showing increased erythrocyte oxygen consumption. It must, however, be emphasized that only part of the thyroid biological effect can be recorded by such measurement. In fact, not less than 60 % of total erythrocyte heat production was derived from the anaerobic pathway in these cells. The activity of membrane bound sodium-potassium ATPase has been proposed to account for a major part of the increased energy demand in thyroid hyperfunction (ref 6). Thus, in animal experiments the activity of the sodium-potassium ATPase in different tissues has been found to be stimulated by thyroid hormones. Monti et al. (ref 7) performed calorimetric studies using ouabain to accomplish inhibition of Na/K ATPase dependent sodium-potassium pump activity in erythrocytes from hyperthyroid subjects. The ouabain-induced change in the heat production rate expressed the energy expenditure connected with the sodium-potassium pump function. A highly significant ($r = 0.84$, $p < 0.001$) positive correlation was found between sodium-potassium pump power and ouabain sensitive sodium transport, thus showing that measurement by direct calorimetry of the sodium-potassium erergy expenditure reliably reflects the sodium-potassium pump function. The total erythrocyte heat production rate was found to be before treatment 131 ± 4 mW/l erythrocytes, significantly higher ($p < 0.001$) than in euthyroid subjects. The corresponding value after treatment was significantly ($p < 0.01$) lower, 112 ± 4 mW/l. The sodium-potassium pump related heat production rate was 11 ± 2 mW/l (8 % of total heat production rate) before and 9 ± 2 mW/l (8 % of total) after treatment, see table 1. These values are not different from those obtained in euthyroid subjects. The data of this work show that the increased heat production rate in erythrocytes from hyperthyroid subjects is not due to increased energy expenditure by the sodium-potassium pump. This indicates that the hypothesis that the increased thermogenesis in hyperthyroidism is due to an increased energy expenditure by the sodium-potassium pump can not be applied to all cells. However, the results of calorimetric studies of the effect of thyroid hormones on erythrocytes and platelets can not be extrapolated without reservation to other cells in view of the fact that human erythrocytes and platelets are without nucleus. In order to have also more representative cells as objects of these observations, Valdemarsson et al. (ref 8) have recently measured heat production rate in lymphocytes from hyperthyroid subjects. Moreover, they have added studies of lymphocyte energy expenditure in patients with subclinical hyper- and hypothyroidism. The latter part of the study was motivated by the fact that it is not known what is the clinical significance of moderate changes in thyroid hormone levels, thus arising difficulties in taking decisions about the treatment

of such patients. The total lymphocyte heat production rate was found to be significantly ($p<0.01$) correlated to the thyroid hormone levels in patients with subclinical hyperthyroidism as well as patients with subclinical hypothyroidism. In the patient group with clinical hyperthyroidism, a significantly increased ($p<0.001$) heat production rate was recorded, see table 2, whereas the patient groups with subclinical hyper- and hypothyroidism had heat production rates not significantly different from the controls.

TABLE 2
Heat production rate in lymphocytes from hyperthyroid patients (Valdemarsson, Ikomi-Kumm and Monti 1990).

	Hyperthyroid	Controls
Number	8	13
Mean value pW/cell	3.43	2.31
SEM	0.25	0.12
	$p<0.001$	

Using the same microcalorimetric technique, Valdemarsson et al. (ref 9) found in a group of hypothyroid patients lower platelet heat production rate than normal, increasing from 51.3 ± 1.6 fW/cell before to 57.1 f/cell after treatment ($p<0.001$), see table 3.

TABLE 3
Heat production rate in platelets from hypothyroid patients before and after treatment (Valdemarsson, Fagher, Hedner, Monti and Nilsson-Ehle).

	Hypothyroid	Controls
Number	10	12
Mean value $\pm$ SEM fW/cell		
before treatment	51.3 ± 1.6	58.8 ± 1.3 $p<0.001$
	$p<0.001$	
after treatment	57.1 ± 1.8	

CONCLUSIONS

1. Energy expenditure is elevated in erythrocytes and lymphocytes from hyperthyroid patients.
2. Thyroid hormones stimulate the erythrocyte metabolic activity along the Embden-Meyerhof anaerobic pathway as well as along the hexose monophosphate aerobic path-way.
3. The increased erythrocyte metabolic rate in hyperthyroidism is not due to increased energy expenditure by the sodium-potassium pump.
4. In hypothyroidism, platelet metabolism is reduced.
5. In subclinical hyper- and hypothyroidism, lymphocyte energy expenditure is normal.
6. Direct microcalorimetry appears to be an adequate method for monitoring net metabolic effects of thyroid hormones at the cellular level.

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