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Calorimetric studies of lymphocytes and hybridoma cells

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Abstract

Total cell metabolic activity can be evaluated easily by measurement of the heat production rate in lymphocytes and hybridoma cells. Standardization of the experimental conditions is necessary in order to obtain reliable results, taking into account the influence on heat production of several parameters, such as calorimetric technique, pH, temperature, cell concentration and anticoagulant used. Useful clinical information has been obtained by microcalorimetric measurements in several diseases: non-Hodgkin lymphoma, chronic lymphocytic leukaemia, thyroid dysfunctions and acromegaly.

INTRODUCTION

Microcalorimetry has been applied in the last two decades to metabolic studies of human blood cells in physiological and pathological conditions. Although erythrocytes are the blood cells which have been the object of most studies, there is good reason to believe that in the future lymphocytes will attract more and more attention. Lymphocytes are nucleated cells and therefore, in comparison to erythrocytes, they are more representative of most cells of the human body. Moreover, lymphocytes play a very important immunological role in many diseases.

The present review deals with microcalorimetric studies on lymphocytes and on hybridoma cells, which are cells obtained by fusion of mouse spleen lymphocytes and myeloma cells.

METHODOLOGICAL STUDIES

A higher heat production rate, 2.7 ± 0.5 pW per cell, was found in human lymphocytes collected from defibrinated blood and suspended in plasma as compared with the corresponding value, 1.8 ± 0.3 pW per cell, when cell suspensions were obtained from heparinized blood. Lymphocytes from heparinized blood gave more reliable (steady state) calorimetric curves [1].

Simultaneous calorimetric measurements on lymphocytes suspended in plasma and in RPMI buffer solution containing sodium bicarbonate did not

show any difference, suggesting that the medium does not influence cell metabolism [1].

Similarly to our previous studies with erythrocytes, we found that lymphocyte heat production rate increases linearly with pH in the range 7.0–7.6 [1]. Lymphocyte heat production rate also shows a linear relationship with temperature in the range 25–37 °C [1].

Storage of lymphocytes in sodium chloride solution for several hours at room temperature did not affect the calorimetric results. When the cells were suspended in plasma, a small decrease (5%) in heat production was noted after 4 hours [1].

Slightly higher heat production per cell was found for lymphocytes in the concentration range $(0.5–4.0) \times 10^6$ cells ml^{-1} compared with the corresponding value when higher cell concentrations were used, thus suggesting a certain degree of "crowding effect" [1]. A similar "crowding effect" was noted for hybridoma cells in the concentration range $(0.64–4.0) \times 10^5$ cells ml^{-1} [2], with lower values at cell concentrations in the higher range.

The calorimetric curves show a steady state appearance when lymphocyte concentrations are kept below 5×10^6 cells ml^{-1}, whereas unstable curves are obtained at higher cell concentrations [1]. A similar effect of cell concentration on the calorimetric curves was noted for hybridoma cells: steady state curves at the concentration range $(0.6–4.5) \times 10^5$ cells ml^{-1} and rapidly descending curves at concentrations above 1×10^6 cells ml^{-1} [2].

BASAL HEAT PRODUCTION

A certain degree of variation has been noted by different investigators who performed calorimetric measurements on human lymphocytes using different experimental conditions. Bandmann et al. [3] recorded with static ampoule calorimeters values of 2.2 ± 1.4 pW per cell for lymphocytes suspended in plasma after preparation from defibrinated blood. Gorski and Levin [4], using flow microcalorimetry, measured values in the range 3–10 pW per cell for lymphocytes suspended in serum + medium 199 after cell preparation from heparinized blood. These variations show that several parameters in the experimental conditions, such as calorimetric technique and anticoagulant used, may affect the results. It thus appears very important to standardize both cell preparation and calorimetric measurement. Heat production rate for hybridoma cells was found to be 30–50 pW per cell [2].

INHIBITION OF CELL METABOLISM

Heat production was measured during inhibition of different metabolic pathways. In hybridoma cells, anaerobic metabolism was inhibited by adding sodium fluoride at variable concentrations. Heat production rate de-

creased by 10–60%, reaching the lowest value at 50 mM sodium fluoride concentration. By adding sodium azide at various concentrations to the hybridoma suspensions, oxidative phosphorylation was inhibited. The greatest inhibition, 55% decrease of heat production rate, was achieved when the inhibitor concentration was 160 mM. Inhibition of aerobic metabolism was also accomplished by adding to the hybridoma suspensions rotenone, which interferes with electron transfer. Maximum decrease of heat production, 40%, was recorded when the inhibitor concentration was 0.1 mM [2]. It seems that addition of sodium azide or rotenone did not achieve complete suppression of aerobic metabolism. In fact, simultaneous measurements of oxygen consumption and heat production in hybridoma suspensions show that aerobic metabolism accounts for 80% of the total metabolic activity [2].

STIMULATION OF CELL METABOLISM

Krakauer and Krakauer [5] were the first to study calorimetrically lymphocytes under stimulation by incubating horse lymphocytes with mitogenic agents. They found increasing heat production rate after 2 days' incubation, reaching maximum effect at 4–5 days. The recorded effect preceded the onset of increased DNA synthesis. Gorski and Levin [4] studied the effect of activation of cultured human lymphocytes with mitogens. A significant increase of heat production was noted after 1–3 days' incubation. Fäldt et al. [6] recorded a slight increase of heat production when human lymphocytes were stimulated with immune complexes.

CLINICAL STUDIES

Non-Hodgkin lymphoma

Heat production rate was measured in lymphocytes from 21 patients with non-Hodgkin lymphoma (NHL) to see if cell metabolic activity was correlated to the degree of malignancy of the disease [7]. Calorimetric measurements showed higher values ($p < 0.005$) for patients with progressive disease as compared with patients who improved during treatment. The mean heat production rate was 3.7 ± 1.1 pW per cell in the former group and 2.4 ± 0.5 pW per cell in the latter group. A recently completed study [8] of a group of 76 NHL patients has confirmed the results of the previous investigation. Median survival for the group of patients with elevated calorimetric values was 8.5 months, whereas for the patients with normal heat production rate the median survival was 39 months ($p = 0.005$). These results show that an elevated lymphocyte metabolism in NHL patients is associated with a higher degree of malignancy, thus suggesting that microcalorimetry can be used to assess the prognosis of the disease. This is particularly important in NHL, taking into account that this disease includes a heterogeneous group of

tumours with variable prognosis; therefore, the optimal type of therapy cannot be chosen without a correct evaluation of the degree of malignancy in individual cases.

Chronic lymphocytic leukaemia

Lymphocyte heat production rate was measured in chronic lymphocytic leukaemia (CLL), the most common type of leukaemia [9]. The purpose of the investigation was to study the unexplained syndrome of hypermetabolism, often affecting CLL patients and causing several symptoms such as loss of weight, fever and sweating. The conclusion of the study was that the increased lymphocyte population in this disease may explain hypermetabolism, despite the fact that heat production per cell was lower than normal.

Thyroid dysfunction

Valdemarsson et al. [10] have measured heat production rate in lymphocytes of patients with thyroid dysfunction. In agreement with previous calorimetric studies on blood cells [11–14], increased values were found in patients with hyperthyroidism, 3.42 ± 0.25 pW per cell, as compared with the corresponding value in the control group, 2.31 ± 0.12 pW per cell ($p < 0.001$). Patients with subclinical hyper/hypothyroidism were found to have normal heat production rate. These results give further support to the results of previous investigations, indicating that microcalorimetry is well suited for studying the influence of thyroid hormones on cell metabolism. This is important in view of the fact that it is clinically difficult to evaluate whether patients with subclinical hyper/hypothyroidism should be treated or not. There are in fact no previous investigations where the effect of slight thyroid dysfunctions on cellular energy expenditure was studied.

Acromegaly

Valdemarsson et al. [15] have measured heat production rate in lymphocytes from patients with acromegaly to ascertain whether calorimetric values could reflect the degree of activity of the disease, which is often difficult to estimate clinically or by traditional laboratory measurements. Acromegaly is a disease with increased production of growth hormone, often caused by tumours of the pituitary gland. This pathological condition is usually treated surgically or by radiotherapy. However, there is a 20% incidence of recurrence of the tumour that is not always easy to ascertain. The results of this calorimetric investigation show that heat production rate in lymphocytes is significantly correlated to the degree of disease activity.

CONCLUSION

Modern microcalorimeters give the possibility of measuring heat production rate in small samples of lymphocytes and hybridoma cells in suspension. The influence on heat production of variations in experimental conditions indicates the importance of standardization of the procedure. Information of clinical interest can be obtained by measuring heat production rate in resting cells, as well as cells in a state of metabolic inhibition or stimulation.

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