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Energetic study of human lymphocytes and the metabolic response to antigenic stimulation ☆

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Abstract

Human peripheral-blood lymphocytes obtained from healthy donors were isolated using Ficoll-paque, Percoll gradients and gelatine beads. Their heat production was measured in the non-activated and activated state under stirred conditions by microcalorimetry and, in parallel, measurements of lactate production and oxygen consumption were performed. Phytohaemagglutinin (PHA) was used for antigenic stimulation of the cells. A heat production rate of about 1 pW per cell was obtained for resting cells obtained after 20 hours blood storage at room temperature. The present calorimetric value for resting cells is in close agreement with the value calculated from the analytical results. The mitogenic response to PHA produced an immediate exothermic signal. The rate of lactate production showed a twofold increase, but the oxygen consumption decreased.

Keywords: Calorimetry; Lymphocytes; Metabolism; Phytohaemagglutinin

1. Introduction

The application of microcalorimetry to the analysis of cellular processes has made an important contribution to life sciences for more than two decades.

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Microcalorimetry allows continuous monitoring of biochemical enthalpy changes of almost any cellular process. Several calorimetric studies on human lymphocytes have been performed. Bandmann et al. [1] obtained a rate of heat production of (2.2 ± 1.4) pW per cell for the plasma suspension of human lymphocytes prepared from defibrinated blood. Ikomi-Kumm et al. [2], using static ampoules in a heat conduction type of microcalorimeter, obtained similar values. However, when using heparin as anticoagulant, decreased heat production rates were observed for human lymphocytes in autologous plasma of (1.7 ± 0.2) pW per cell and (1.6 ± 0.2) for lymphocytes in buffer.

Flow microcalorimetry was applied by Gorski and Levin [3] for metabolic studies of human lymphocytes at rest and during activation with mitogens. A basal heat production of 3–10 pW per cell was obtained and a significant response in heat output was observed 24 h after stimulation with both phytohaemagglutinin (PHA) and purified protein derivative of tuberculin.

The aim of the present work is to analyse thermochemically the basal metabolism of human lymphocytes as well as the metabolic changes induced by mitogenic stimulation with PHA.

2. Experimental

2.1. Isolation of lymphocytes

Blood from six healthy donors at the University Hospital of Lund was collected in plastic bags containing citrate-phosphate-dextrose (CPD) solution. Buffy coats were obtained by direct centrifugation. The collected blood samples were stored for 20 h at room temperature prior to isolation of the lymphocytes. The buffy coats containing mainly white blood cells and thrombocytes were diluted 1:4 with phosphate-buffered saline (PBS) containing 25 IE heparin per ml. Monocytes and lymphocytes were separated from the buffy coat using density gradient centrifugation (1700 rpm for 30 min) of Ficoll-paque (Pharmacia, Uppsala) according to Böyum [4].

Contaminating thrombocytes were removed from the mononuclear cell suspension using a Percoll gradient (900 rpm for 15 min) (Pharmacia, Uppsala) [5]. Contaminating erythrocytes were lysed by a 0.83% buffered ammonium chloride solution. The lymphocytes were separated from the monocytes using a column containing gelatine beads [6]. The purified lymphocytes were washed twice with PBS, resuspended in 2 ml of RPMI 1640 medium containing 20 mM HEPES buffer (Flow Laboratories, Rockville, MD, USA) and incubated at 37°C for 30 min under gentle shaking prior to experiments.

A stock cell suspension of 2×10^6 cells per ml was prepared in RPMI 1640 medium supplemented with 10% heat-inactivated and dialysed [7] foetal calf serum, 0.05 g l^{-1} gentamicin and 0.3 g l^{-1} glutamine. Viable cell counts were carried out using the Trypan blue exclusion method [8]. Mitogenic stimulation of the lymphocytes was performed by addition of PHA (Sigma, USA) from a stock

solution of 6.4 mg ml^{-1} PBS to a final concentration of 20 μg ml^{-1} of cell suspension.

2.2. Microcalorimetry

A twin heat-conduction microcalorimeter 2277 TAM (Thermometric AB, Järfälla, Sweden) equipped with stirred perfusion vessels, was used in all calorimetric experiments [9,10]. The thermal power was measured at 37°C. A small turbine stirring device at a rate of 90 rpm [10] prevented the cells from sedimentation. Samples of 2.7 ml were transferred to the microcalorimeter vessel which subsequently was introduced into the calorimeter. At steady state, PHA was injected into one of the samples to activate the lymphocytes. The rate of heat production was read at 4 h. The baseline value was determined with 2.7 ml of water in the ampoule. In separate experiments, 2.7 ml of the medium was introduced into the calorimeter and the heat production as well as the heat effects following injection and dilution of PHA and of PBS into the medium were determined.

2.3. Determination of oxygen consumption and lactate production

The oxygen consumption was measured in a thermostat-regulated oxygraph (Hansatech, Norfolk, UK). Samples were taken from the stock cell suspension and two consecutive measurements of oxygen consumption were made. The oxygen consumption of the mitogenic stimulated lymphocytes was commenced after incubation for 2 h with PHA. Oxygen consumption by the medium was determined separately and was subtracted from measurements of cellular suspensions.

Lactate production measurements were performed in 10-ml Erlenmeyer flasks each containing 2 ml cell suspension, incubated in a shaking water both at 37°C with and without PHA. The amount of lactate produced was measured enzymatically on deproteinized and neutralized samples taken at intervals of 1 h during a period of 4 h [11]. All reactants for the enzymatic assay were from Boehringer Mannheim Scandinavia (Bromma, Sweden).

3. Statistics

Numerical values are given as mean ± standard deviation (SD) and coefficient of variation (CV). The student's t-test was used to determine significance. Linear regression was used for kinetic measurements.

4. Results

In the present work the average heat production rate of the medium amounted to 1.6 μW and was subtracted from the experimental values. The calorimetric experiments gave a mean value of (0.99 ± 0.17) pW per cell with a CV of 12% (Table 1). The rate of lactate production (dn_{lac}/dt) and of oxygen consumption (dn_{O_2}/dt) are listed in Table 2. The corresponding power values were calculated by multiplying

Table 1
The calorimetrically determined thermal power per cell values (P_{cal}) and the calculated power values (P_{lac} and P_{O_2}) obtained from the rate of lactate formation and oxygen consumption measurements based on glucose catabolism. Figures in parentheses denote the number of determinations

Thermal power	Non-activated		PHA-activated	
P_{O_2}/pW	0.70 ± 0.12	(12)	0.54 ± 0.09	(5)
P_{lac}/pW	0.27 ± 0.08	(6)	0.56 ± 0.12	(5)
$P(P_{O_2} + P_{lac})$/pW	0.97 ± 0.18		1.10 ± 0.18	
P_{cal}/pW	0.99 ± 0.14	(17)	1.56 ± 0.12	(5)

Table 2
The rate per cell values of lactate formation and oxygen consumption for the resting and activated human lymphocytes. The cell concentration was in the order of 2×10^6 cells per ml. Figures in parentheses denote the number of determinations

	dn_{lac}/dt in (amol s^{-1})		dn_{O_2}/dt in (amol s^{-1})	
Non-activated	3.52 ± 1.00	(6)	1.67 ± 0.67	(6)
PHA-activated	7.27 ± 1.56	(5)	1.14 ± 0.18	(5)

the rate values with the corresponding molar enthalpy changes. Anaerobic lactate production from glucose is associated with a molar enthalpy change of -109 kJ per mol of glucose [12].

The oxygen consumption power values were obtained using the average molar enthalpy change of -450 kJ per mol of oxygen [13,14]. To these values was added the molar enthalpy change of the HEPES buffer reaction, -22.52 kJ mol^{-1} [15]. The calorimetrically measured power values and analytically determined power values based on glucose catabolism are listed in Table 1.

4.1. PHA-stimulation of lymphocytes

A typical calorimetric recording for both stimulated and non-stimulated lymphocytes is shown in Fig. 1. Significant increases in the thermal power ($p < 0.001$) as well as in the rate of lactate production ($p < 0.001$) are observed during stimulation with PHA. Results of stimulation experiments are summarized in Tables 1 and 2. A twofold increase in lactate production was obtained as a result of PHA stimulation. Lymphocytes measured 2 h after addition of PHA showed a decrease in oxygen consumption ($p < 0.006$) compared with non-stimulated lymphocytes. Heat production of the suspension medium was not affected by addition of PBS or PMA.

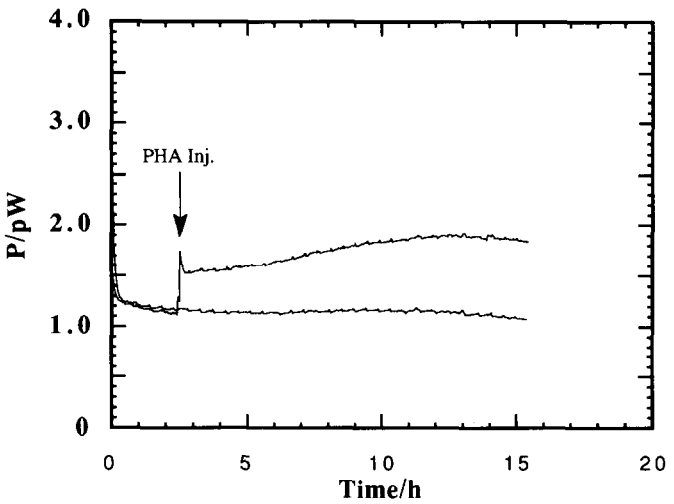

Fig. 1. Thermal power per cell for human lymphocytes in resting and activated state. The cell concentration was 2.2×10^6 per ml. The arrow indicates the time at which PHA was added to a final concentration of 20 μg ml^{-1}.

5. Discussion

The primary objective of this study was to characterize thermochemically the basal metabolism of lymphocytes and to evaluate the relative responsiveness of lymphocytes to the plant lectin PHA. The cell separation techniques used are well established and efficient in yielding highly purified lymphocytes. The use of gelatine beads in the separation of the phagocytic cells was described previously by Sjögren et al. [6]. The method allows convenient handling of a large number of cells. The purified material is obtained in a good yield and at a high viability. Measurements on living cells are usually carried out in plasma, buffer or an appropriate medium.

It is therefore important to determine the heat flux (due to, for example, viscosity changes or spontaneous oxidation processes) of the medium and correct for it. The value obtained in the present work is (0.99 ± 0.17) pW per cell, which is almost half the value obtained by Ikomi-Kumm et al. [2]. In that work the lymphocytes were not stored before starting the preparation process. In the present study the cells were stored for about 20 h before purification which probably caused a decrease in their metabolic activity [2].

From the data obtained, about 25% of the total heat production originated from the conversion of glucose to lactate and the rest from glucose oxidation. The sum of analytically determined thermal power values is in full agreement with the calorimetrically measured power values. It has been demonstrated that a cascade of different biochemical reactions are initiated immediately upon the addition of mitogen to lymphocyte cultures [16]. One of them is protein synthesis which begins 2 h after mitogen stimulation and accelerates during the first 12 h of culture [16].

These findings are in line with the present calorimetric results where we observed an increase in the heat production rate following the addition of PHA. However, they are contradictory to the results by Gorski et al. [3] who found a decrease in heat output during the initial phase of mitogenic activation of human lymphocytes.

Ross and Loos [17] have investigated the short- (0–4 h) and long-term (>4 h) effect of PHA on lymphocytes. In the short-term experiments, either glycolysis or the Krebs cycle can provide the energy necessary for the processes initiated by PHA, while in the long term both metabolic pathways are active. They found that PHA induced an increase in lactate and pyruvate production. They concluded that the energy demand is met by increasing glycolysis and thereby Krebs cycle induction after two hours. Thus, the twofold increase in lactate formation corresponds to the increase in glycolysis but the decrease in oxygen consumption could be either the direct effect of PHA or, possibly, the limiting of diffusion of oxygen as a result of cellular aggregation [18].

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