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A comparison of the calorimetric analysis of granulocyte activation by flow and batch systems

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Abstract

Granulocyte activation by phorbol myristate acetate (PMA) is a model with which to study phagocytosis. Measurement of heat production during cell activation gives a parameter that can be used to make a quantitative evaluation of the functional potential of these phagocytizing cells. In the present study, the heat production in PMA-activated granulocytes was measured using batch calorimetry. The batch technique is simpler and requires a sample volume of 2 ml, corresponding to 1/60 of that necessary with the flow technique.

INTRODUCTION

The availability of advanced instrumentation has led to the application of biophysical methods to the analysis of cellular processes. Microcalorimetry allows direct and continuous measurement of cell metabolism and gives the fundamental physicochemical value of the enthalpy changes associated with the metabolic processes [1]. In the last 20 years, calorimetric studies on blood cells have been conducted in several laboratories [2,3] and the technique has been successfully applied to the measurement of heat production by activated granulocytes [4–12]. A new parameter reflecting the functional potential of these cells while carrying out phagocytosis was thus established. In a previous study [4], using flow calorimetry, heat production was correlated to other parameters expressing aerobic (O_2 consumption, CO_2 production) and anaerobic (lactic acid production) metabolism. In the present study ,we introduce a simple concept for batch calorimetry with the intention of reducing the sample requirement and

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increasing the ease of operation. The aim of the present study was to measure heat production during granulocyte PMA activation using batch calorimetry and to compare the results obtained with those previously recorded with flow calorimetry.

MATERIALS AND METHODS

Cell preparation

Venous blood was collected from healthy donors into tubes containing citrate phosphate dextrose (CPD) solution in a ratio of 9:1. The granulocytes were obtained by dextran sedimentation followed by density gradient separation using Isopaque–Ficoll [13]. After hypotonic lysis of erythrocytes, the granulocytes were suspended in Gey's buffer [14], pH 7.4, which had been depleted of calcium and magnesium salts. The cell concentration was 1.25×10^6, 2.5×10^6, 5.0×10^6 and 10.0×10^6 cells ml^{-1} respectively.

Batch calorimetry

The calorimeter was a 2277 TAM from Thermometrics AB, Sweden [15]. Glass ampoules containing 2 ml of the cell suspension were used. A cannula was inserted through the rubber septum, sealed with silicone grease and connected to a syringe containing 0.2 ml of PMA solution. At thermal equilibrium, compression of the syringe forced the silicon plug out of the end of the cannula and thereby allowed mixing of the two solutions. Complete homogeneity was achieved through double movement of the syringe piston. The heat evolved or adsorbed was calculated by integrating the areas of the power–time curves obtained. Calibration of the instrument was performed electrically. The heat of dilution of both solutions was measured in parallel and subtracted from the measured energies.

Other measurements

The kinetics of O_2 consumption were measured with a Clark electrode in a Yellow Spring system (Yellow Spring Instrument Co, Ohio). Glucose catabolism through the hexose monophosphate shunt (HMPS) was evaluated by measuring the CO_2 produced during oxidation of (1-^{14}C)-glucose and (U-^{14}C)-glucose. In the instrument utilized (Bactec, Johnston Laboratories, Cockeysville, MD) the CO_2 evolved was collected in an ionization chamber and measured with a vibrating-reed electrometer. Lactic acid levels were determined enzymatically with commercially available kits from Boehringer, Mannheim, Germany. The pH measurements were performed using a pH electrode from Orion Res. Inc., MA.

RESULTS

A sharp increase in power was observed after addition of the chemical inducer to the human granulocytes, as illustrated in Fig. 1. The maximum was reached after 15–18 min, after which time the thermal power rapidly declined to baseline values after about 70–90 min. In the batch calorimeter, the baseline includes the basal heat production of the cells (Fig. 1A). Therefore the return to the original basal value indicates that the cellular metabolism has switched from the activated to the steady state condition. The kinetics of activation observed in the two instruments were slightly different. In the batch power–time curves we rarely encountered a biphasic slope, whereas such curves were frequently noted when the flow system was used [4]. In the batch experiments carried out at various cell concentrations, the total thermal energy released upon activation was linearly de-

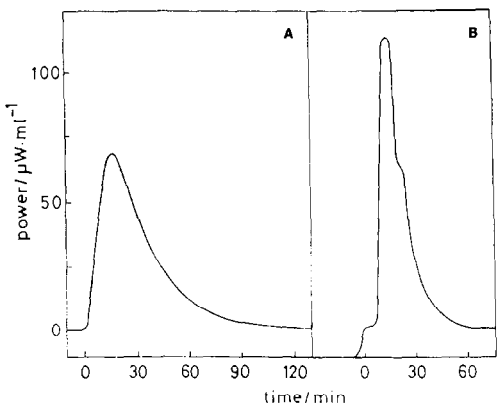

Fig. 1. Power–time curves from single experiments of human granulocytes (5×10^6 cells ml^{-1}) activated with 100 ng ml^{-1} of PMA added at zero time; A, batch calorimeter measurements; B, flow calorimeter measurements [4].

TABLE 1

Energy effects produced by different concentrations of granulocytes activated in a batch calorimeter by the addition of 100 ng ml^{-1} of PMA: the values (±SD) were calculated from duplicate determinations carried out on blood from three different donors

Granulocytes per millilitre ($\times 10^{-6}$)	Q per millilitre (mJ ml^{-1})	Q per granulocyte (nJ per cell)
1.25	57.8 ± 4.5	46.2 ± 3.6
2.5	114.3 ± 11.5	45.7 ± 4.6
5.0	146.2 ± 13.2	29.2 ± 2.6 [a]
10.0	225.0 ± 26.1	22.5 ± 2.6

[a] In flow experiments, values of 23 nJ per cell were found [4].

TABLE 2

Thermochemical and biochemical results from PMA-activated granulocytes (2.5×10^6 cells ml^{-1}) in a batch calorimeter; mean values (±SD) were calculated per cell from eight different duplicate experiments and refer to the period of activation (120 min)

Q experimental	45 ± 9 nJ
CO_2 produced	90 ±12 fmol
O_2 consumed	92 ±12 fmol
Q_1 calculated	45 ± 6 nJ
Lactic acid	5 ± 2 fmol
Q_2 calculated	0.3± 0.1 nJ

[a] The ΔH values used in calculations of the aerobic Q_1 and anaerobic Q_2 metabolisms were −488 and −58.5 kJ mol^{-1} respectively [16,17].

pendent on cell density up to 2.5×10^6 cells ml^{-1}. At higher cell densities, a markedly reduced response of thermal energy was observed (Table 1).

The data presented in Table 2 were obtained from eight experiments where simultaneous batch measurements of heat production as well as O_2 consumption, and CO_2 and lactic acid production from activated granulocytes (2.5×10^6 cells ml^{-1}) were carried out. No increase in anaerobic glycolysis, measured in terms of lactic acid production, was observed in PMA-activated granulocytes. The production of $^{14}CO_2$ during oxidation of ^{14}C-glucose was stoichiometrically proportional to the moles of oxygen consumed. We derived a value of −488 kJ per mole O_2 (Table 2) in agreement with the predicted enthalpy change, corresponding to the number of aerobic oxidized moles of glucose and proportional to the consumed oxygen.

DISCUSSION

Previous studies carried out using a flow calorimeter indicated that thermochemical measurements could be successfully utilized to study the metabolism of activated granulocytes [4]. Some difficulties were encountered with the previous instrumentation: high flow rates were required to reduce adhesion of the granulocyte to the flow line, and unstable instrumental baselines and clogging of the line itself were experienced. Moreover, large quantities of granulocytes were required for the measurements. All these considerations prompted us to use a batch calorimeter. The amount of cellular suspension required was reduced from 60 to 2 ml for each experiment. The environmental conditions of a batch instrument are completely different from those of a flow line in which the cells are continuously agitated and well aerated. In the batch system, the crowding effect, as shown in Table 1, illustrates the decrease in thermal energy produced by an increased cell concentration [18]. In the flow experiments, it was found that the power produced by the phagocytosis, during the

metabolic burst, corresponded to the enthalpy change of the aerobic glucose metabolism. We therefore reinvestigated the correlation between the heat produced by the granulocytes and the calculated enthalpy change in their metabolism. In the batch experiments, the heat produced was in accordance with that predicted by the enthalpy change for glucose oxidation to CO_2 and H_2O in aqueous solution [16]:

$$\text{Glucose} + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (1)$$

with

$\Delta H = -488$ kJ per mole O_2 [16].

However, other authors use a slightly smaller value of ΔH for reaction (1), -478 kJ per mole O_2 [1]. In any case, applying this value, there is fairly good agreement between our calculated and experimental data. This agreement is much better than that obtained in the flow experiments, where the CO_2 recovered accounts for only 80% of the oxygen consumed [4]. The explanation for the good agreement is probably that the calculated values were based on the enthalpy change for glucose oxidation which in granulocytes is carried out almost exclusively in the HMPS, mitochondria being almost absent in mature granulocytes. Moreover, glucose catabolism through the HMPS is coupled to enzymatic oxidation of the reduced coenzyme and the water produced, without any accumulation of energy-rich intermediate metabolites at the end of the experiments [18]. Anaerobic glycolysis, which has a low enthalpy value, does not seem to contribute to the heat production of granulocyte activation. In fact, there was no increase in lactic acid production when granulocytes were stimulated with PMA. The results previously obtained with the flow calorimeter system have been confirmed in this study carried out with batch calorimetry. The slight differences observed when comparing the two calorimetric techniques might be ascribed to the different time constants of the calorimetric vessels, permitting a more rapid recording of activated granulocytes in the flow system. The batch system, however, is easier to operate and requires significantly less material, thus offering an alternative calorimetric method to study phagocytic capabilities of leucocytes.

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REFERENCES

1 J.P. Belaich, Growth and metabolism in bacteria, in A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, pp. 1–42.

2 M. Monti, In vitro thermal studies of blood cells, in A.M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, Wright, Bristol, 1987, pp. 131–146.
3 K. Levin, Studies on blood cells, in A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, pp. 131–143.
4 C. Eftimiadi and G. Rialdi, Increased heat production proportional to oxygen consumption in human neutrophils activated with phorbol 12-myristate 13-acetate, Cell Biophysics, 4 (1982) 231–244.
5 C.Eftimiadi and G. Rialdi, Increased energy expenditure by granulocytes during phagocytosis of *Staphylococcus aerus* compared to other staphylococci, Infect. Dis., 150 (1984) 366–371.
6 C. Eftimiadi and G. Rialdi, A new calorimetric technique to analyze bacteria–phagocyte interactions, Microbiologica, 8 (1985) 297–301.
7 C. Eftimiadi and G. Rialdi, Calorimetric analysis of phagocytosis of staphylococcal protein-A/IgG complexes, Microbiologica, 8 (1985) 255–261.
8 C. Eftimiadi and G. Rialdi, Calorimetric and chemiluminescence studies on human neutrophils, Thermochim. Acta, 85 (1985) 489–492.
9 M. Monti, R. Fäldt, J. Ankerst and I. Wadsö, A new approach to detection of antigen–antibody complexes by microcalorimetric measurements of heat production in blood cells, J. Immunol. Methods, 37 (1980) 29–37.
10 R. Fäldt, J. Ankerst, M. Monti and I. Wadsö, Heat production in different populations of human blood cells exposed to immune complexes in vitro, Immunology, 46 (1982) 189–198.
11 R. Fäldt, J. Ankerst and M. Monti, Heat production rate in polymorphonuclear granulocytes from patients with acute myelogenous leukaemia and healthy individuals, Br. J. Haemat., 58 (1984) 671–678.
12 J. Ankerst, R. Fäldt and M. Monti, Decreased responsiveness to immune complexes of granulocytes from patients with acute leukemia in remission demonstrated by microcalorimetry, Leukemia Res., 8 (6) (1984) 997–1002.
13 A. Böyum, Separation of leucocytes from blood and bone marrow, Scand. J. Clin. Lab. Invest., 21 (1968) 1–109.
14 G.O. Gey and M.K. Gey, The maintenance of human normal cells and tumor cells in continuous culture, Am. J. Cancer, 27 (1936) 45–76.
15 J. Suurkuusk and I. Wadsö, A multichannel microcalorimetry system, Chem. Scr., 20 (1982) 155–163.
16 M. Monti and I. Wadsö, Calorimetric studies on blood cells, in M.N. Jones (Ed.), Biochemical Thermodynamics, Elsevier, Amsterdam, Vol. 1, 1979, pp. 256–280.
17 R.C. Wilhoit, Selected values of thermodynamic properties, in H.H. Brown (Ed.), Biochemical Microcalorimetry, Academic Press, New York, 1969, pp. 305–317.
18 V. Essman, Effects of cell concentration on the metabolism of normal and diabetic leucocytes in vitro, Metabolism, 13 (1964) 354–360.
19 P. Dri, P. Bellavite, G. Berton and F. Rossi, Interrelationship between oxygen consumption, superoxide anion and hydrogen peroxide formation in phagocytosing guinea pig polymorphonuclear leucocytes, Mol. Cell. Biochem., 23(2) (1979) 109–115.