Thermochimica Acta, 172 (1990) 53–60
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

APPLICATION OF MICROCALORIMETRY TO THE STUDY OF LIVING CELLS IN THE MEDICAL FIELD *

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SUMMARY

In the last 20 years we have applied microcalorimetry to the study of metabolism in living cells with the purpose of obtaining useful information in the medical field. Both cell suspensions and pieces of tissues have been used. Cell metabolism, as recorded by heat production rate, has been found to reflect the activity of the disease in several pathological conditions: thyroid dysfunctions, acromegaly, acute leukaemia, peripheral arterial disease. In non-Hodgkin lymphoma, a heterogenous group of tumors with variable degree of malignancy, high heat production rate in tumor cells and lymhocytes was associated with a poor prognosis. Microcalorimetry appears to be a useful methodology in the medical field.

INTRODUCTION

Since 20 years we have applied microcalorimetry to the study of metabolism in living cells. Most of the work has been done with human material but also other mammalians have been used, mainly in projects where it was necessary to sacrifice the animals in order to obtain the desired specimens.

Initial studies

The human erythrocyte was the first cell studied (ref 1-2) and the methodological work done on it was used as a model for the following investigations on other cells. The initial part of the work was concentrated on studying the various parameters affecting the calorimetric results: type of calorimeter, suspension media, preparation of cell suspensions, pH, temperature. Heat production rate was found to increase linearly in the physiological range by 1.2 % per 0.01 pH unit whereas the temperature coefficient for heat production was determined to be $Q_{10} = 2.8$ in the temperature range 32 - 42 ^{o}C. Similar studies were later performed for lymphocytes (ref 3) platelets (ref 4) adipocytes (ref 5) and skeletal muscle (ref 6).

In the early stage of our work with blood cells we have been concerned whether the preparation of the suspensions might affect the metabolic state of the cells. In order to

* Presented at the 7th International Symposium on Microcalorimetric Applications in Biology, Egham, U.K., 9–11 April 1990, and Dedicated to Ingemar Wadsö on the Occasion of his 60th Birthday.

0040-6031/90/$03.50

investigate this possibility, we measured simultaneously heat production in samples of whole blood and the different purified cell fractions and plasma (ref 7). We found full agreement between the values obtained in whole blood and the values resulting from the sum of the heat production by the single blood constituents.

In one of the first clinical applications of this methodology we found that heat production rate in erythrocytes from patients with anemia was significantly higher than the corresponding value in healthy subjects (ref 8). Our interpretation of these results is that anemia stimulates the bone marrow to increased production of new erythrocytes to compensate for the deficiency, resulting in an increase of young cells, with higher metabolic activity, in the circulation.

Thyroid dysfunction was one of the first clinical conditions investigated and detailed information is given by Monti et al. (ref 9).

MEASUREMENT OF HEAT PRODUCTION DURING INHIBITION OR STIMULATION OF CELL METABOLISM

Inhibition

By use of ouabain, that blocks completely the sodium-potassium pump, decreased heat production was recorded (ref 10-11). These changes were correlated to variations of intracellular sodium (ref 12) and indication was obtained that the calorimetric values reflected the function of the sodium-potassium pump. This experimental model was used to study calorimetrically the importance of the sodium-potassium pump in obesity (see later in the present article) and hyperthyroidism (ref 9).

The energy flow via the aerobic and anaerobic pathway respectively was estimated by comparing heat production with both pathways in operation and simultaneously in the presence of sodium fluoride (ref 13), that inhibits the Embden Meyerhof pathway, or sodium azide and rotenone (ref 14), that inhibit the aerobic metabolism. This model was applied in the clinical study of hyperthyroidism (ref 9).

Stimulation

Erythrocyte metabolism has been studied by microcalorimetry (ref 15) during stimulation of the pentose phosphate pathway with methylene blue, maximal effect being obtained at concentrations of 10^{-4} M. It is known that only minor quantities of substrate are metabolized in the erythrocytes through the aerobic pathway, however, this metabolic pathway has been considered to be of importance in some pathological conditions. A large increase of heat production rate, 6-7 times, was observed in the cells stimulated as compared to the corresponding values in unstimulated cells. In an application of this model to clinical studies, we found increased stimulation values in erythrocytes from patients with renal failure (ref 16) and normal values in patients with liver diseases (ref 17). These results are in disagreement with previous reports suggesting that haemolytic anemia among these patients is related to decreased activity of the pentose phosphate pathway.

The heat of phagocytosis was studied by measuring heat production rate in granulocytes exposed to immune complexes formed in vitro (ref 18-19). In an application of this model to clinical studies, we found that the responsiveness of granulocytes from patients with acute leukaemia to immune complexe stimulation was decreased in the acute stage of the disease and during the first 6 months, whereas after 6 months of treatment the values returned to normal (ref 20). However, the metabolic activity of unstimulated granulocytes was found to be increased in patients with acute leukaemia (ref 21).

In a recent study we have studied the blood biocompatibility of artificial surfaces by measuring the stimulation heat of granulocytes exposed to different polymer materials (see later in the present article).

NON-HODGKIN LYMPHOMA

Non-Hodgkin lymphoma is a heterogenous group of tumors with variable degree of malignancy and therefore great variation in the clinical course of the disease. A correct estimation of the degree of malignancy in the single case is very important in order to choose the best form of treatment. The usual way to make such an evaluation is by the clinical appearance and by the morphology of the tumor cells. However, there is not a complete agreement between such an estimation and the development of the disease in the single cases. There is therefore need for other parameters to improve the estimation of the degree of malignancy. Microcalorimetry was used to study the correlation between the clinical course of the disease and overall metabolism of tumor cells and blood lymphocytes (ref 22). In a group of 21 patients, the heat produced by tumor cells was significantly higher, 5.5 pW/cell (mean value), in the sub-group with progressive disease, as compared to the corresponding value, 3.1 pW/cell, in patients who responded to treatment. Even heat production in blood lymphocytes was found to be higher in the first group. In a later work (ref 23) including 36 patients, it was found that for 20 subjects with lymphoma of high grade malignancy the median value of heat production rate in tumor cells was 3.9 pW/cell whereas for 16 patients with low grade lymphoma the corresponding value was 2.8 pW/cell. A high correlation was found between heat production and patient survival. In comparison to the usual prognostic variables, such as patients age, clinical stage and morphologic grade of malignancy, the calorimetric value was found to be a more reliable prognostic parameter. In a recent work (ref 24), heat production rate was measured in blood lymphocytes from 76 non-Hodgkin lymphoma patients. Median survival was 39 months for patients with normal calorimetric values and 8.5 months for patients with elevated heat production ($p < 0.005$). The prognostic significance of calorimetric values was found even in this group of patients to be superior to the usual prognostic variables. In conclusion, the results of these investigations in non-Hodgkin lymphoma show the clinical usefulness of microcalorimetry in this disease, indicating an association between high heat producion rate in tumor cells and blood lymphocytes, and a poor prognosis.

ACROMEGALY

Acromegaly is a clinical condition characterized by an increase in size of hands, feet and jaws and other less typical symptoms. The disease is due to an excess of growth hormone caused by tumors of the hypophysis. The evaluation of activity degree of the disease is important in order to decide if treatment, operation or irradiation of the hypophysis, has been successful or further therapy is needed. In an attempt to develope a new parameter for evaluation, until now unsatisfactory, of the degree of activity of the disease, we have measured heat production rate in lymphocytes from 15 acromegaly patients. The calorimetric values were found to be higher than normal ($p<0.01$) and significantly correlated to the degree of activity of the disease from a clinical point of view (ref 25).

OBESITY

Most cases of obesity are due to excess of caloric intake but for a sub-group of patients the cause of overweight is unclear. With the support of several experimental studies, the hypothesis has been advanced that decreased cellular metabolic efficiency could play a role in some cases of obesity. A microcalorimetric method has been developed (ref 5) to estimate adipocyte overall metabolism. Adipocyte heat production rate was found to be lower ($p<0.001$) in obese than in lean subjects (ref 26), thus giving support to the hypothesis that a decrease of cellular metabolism might contribute to development of obesity.

By several investigators the suggestion has been advanced that reduced activity of the sodium-potassium pump might be the cause of decreased energy expenditure. We have therefore evaluated in obese subjects the importance of the sodium-potassium pump by quantifying calorimetrically the changes of overall erythrocyte metabolism induced by specific inhibition of the sodium-potassium pump with ouabain (ref 10). No difference was noted between the obese and the normal group. In neither group there was a correlation between ouabain inhibitable rate of metabolism and body weight. Thus, the results of this study do not give support to the hypothesis that the sodium-potassium pump is of importance in the development of obesity. In order to further investigate the importance of cell energy balance in obesity, we have measured heat production rate in adipocytes from obese subjects before and after weight reduction, accomplished by decreased caloric intake through gastroplasty (ref 27). The initial value of heat production was lower than normal, in agreement with our previous study (ref 26). The corresponding value after weight reduction was significantly higher than the first measurement before treatment. These results suggest that the decreased heat production found in adipocytes from obese individuals is a phenomenon secondary to overweight rather than a cause of obesity. However, this phenomenon might be an important cause of accentuation of the obese state.

ANOREXIA NERVOSA

Anorexia nervosa is a psychiatric disorder characterized by severe preoccupation about food of the patients who refuse to eat to the extent of reaching a state of extreme weight

loss. The cause of the disease is unknown and the pathophysiological derangement of energy balance is unclear. In order to reach a better understanding of this pathological condition, we have studied skeletal muscle and platelet thermogenesis in a group of patients with anorexia nervosa (ref 28). Muscle heat production was found to be significantly decreased, by 50 % compared to the corresponding value in healthy subjects. Although platelet heat production was decreased ($p<0.02$) to a lesser degree, it may be associated with disturbances of cellular functions, such as aggregation and adhesivity, known to be energy requiring processes; in fact, bleeding tendency has been observed occasionally in patients with anorexia nervosa.

INFLUENCE OF BETA-ADRENOCEPTOR DRUGS ON SKELETAL MUSCLE METABOLISM

Beta-adrenoceptor blocking agents belong to a very important group of drugs in human medicine, commonly used in several cardiovascular diseases. It is a well known clinical observation that patients treated with these drugs may be affected by the undesirable side effect of fatigue. Despite much research, the mechanism behind this symptom is still unclear. We have investigated thermogenesis in human skeletal muscle by direct microcalorimetry in order to find out whether beta-adrenergic drugs cause a decrease of cell metabolism leading to decreased ATP production and therefore reduced muscle function. In the first investigation (ref 29) we have measured heat production rate and muscle contractile performance before and after random administration of three beta receptor antagonists with different pharmacodynamic properties: propranolol, atenolol and pindolol. Our results suggest that a selective beta-receptor drug like propranolol, but not the other two drugs, causes a decrease of thermogenesis and muscular contraction. In a second work (ref 30) we could record decreased skeletal muscle thermogenesis also after administration of a selective drug, metoprolol, if the subjects were exposed to acute stress. The decrease of heat production rate in these subjects, 0.3 mW/g muscle, was found to be of the same magnitude as the decrease of body temperature calculated by means of a thermistor in an indwelling pulmonary arterial catheter (ref 31).

UREMIA - HEMODIALYSIS

The pathogenesis of muscular dysfunction in uremia is unknown. Several authors have suggested that carnitine deficiency might be the cause. We have evaluated muscle metabolism by measurement of heat production in a group of 28 hemodialysis patients and related the results to muscle function, thyroid hormones, lipoproteins and carnitine levels (ref 32). Both muscle heat production and function were found to be reduced. However, there was no indication that carnitine deficiency was the cause of it. In agreement with previous studies, we found subnormal thyroid hormone levels, that correlated significantly with heat production rate. Insufficient oxigenation of muscle tissue due to anemia might be of importance in the development of decreased muscle metabolism/function in these patients. In fact, a positive correlation was observed between hemoglobin concentration and muscle heat production (ref 33).

PERIPHERAL ARTERIAL DISEASE

Arteriosclerosis leading to stenosis in the vessels of the lower limbs is a common disease. It is important to make in these patients a quantitative evaluation of the consequences in the peripheral tissue of decreased oxigenation secondary to impairment of blood flow. This is, however, not accomplished satisfactorily with conventional methods. We have therefore used microcalorimetry to quantify the derangement of muscle metabolism in patients with variable degree of peripheral arterial insufficiency (ref 34). Decreased heat production was found in patients with severe arterial insufficiency. Moreover, a positive linear correlation was noted between calorimetric values and leg blood flow.

DIABETIC CARDIOMYOPATHY

During many years cardiac involvement in patients with diabetes mellitus has been thought to be due exclusively to coronary atherosclerosis, known to occur with higher frequency in diabetic than in non-diabetic subjects. From medical reports in the last decade there is increasing evidence that diabetic cardiomyopathy is not necessarily a consequence of coronary disease. We have studied by microcalorimetry myocardial tissue metabolism in rats with diabetes (ref 35). Lower rates of heat production were found in comparison to the corresponding values in healthy rats. Our observation gives support to the hypothesis that a derangement of myocardial metabolism independent of coronary disease might occur in diabetes mellitus.

TOXICOLOGY

By microcalorimetry we have evaluated the biocompatibility of polymer membranes used in hemodialysis (ref 36). Granulocyte suspensions were enclosed in calorimeter ampoules lined with polyetherpolycarbonate or cuprophan, whereas ethylene propylene was used as reference material. Heat production was measured in resting cells and during stimulation of phagocytosis by zymosan. The degree of phagocytic activation was found to be related to the degree of biocompatibility, thus indicating that microcalorimetry offers a new tool for the evaluation of biomaterials.

By microcalorimetry we have investigated the effects on cell metabolism of various substances of interest in human medicine: caffeine (ref 37-38), other xanthine derivatives and adenosin receptor agonists (ref 39), calcium antagonists (ref 40), gentamicin (ref 41), cadmium and lead (ref 42).

THYROID DISORDERS

See Monti et al (ref 9).

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