MICROCALORIMETRY OF ISOLATED MITOCHONDRIA FROM BROWN ADIPOSE TISSUE

Effect of guanosine-di-phosphate

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Received 9 January 1979

1. Introduction

Few authors have estimated heat output from isolated mitochondria using microcalorimetry, and no such data have been reported about brown fat mitochondria [1–4] the main function of which is heat production. Recently a microcalorimetric study of isolated cells from brown adipose tissue has been reported leading to the conclusion that the thermogenic contribution of this organ in hamsters is low [5]. Due to the technical difficulties posed by the isolated-cell preparation, we felt it was of interest to demonstrate that similar measurement made on uncoupled, isolated mitochondria (a simpler technique) yielded similar conclusions.

We have found that a good correlation exists between estimates of heat output calculated from oxygen consumption and those obtained with direct microcalorimetric assay of isolated mitochondria. The limitation of energy dissipation by guanosine-di-phosphate (GDP) (a recoupling agent) in these mitochondria could be directly observed.

2. Materials and methods

Week 6 Sprague-Dawley male rats (100 g body wt) were used. Control animals were bred at 25°C, where as cold-adapted animals had been exposed to 6°C from week 3 to week 6.

Mitochondria were prepared as in [6]. A Clark oxygen electrode (Gilson apparatus) was used to measure oxygen consumption (25°C). The incubation medium contained: 100 mM KCl, 20 mM K-TES (pH 7.2), 2 mM EDTA, 2 mM $MgCl_2$, 10 mM KH_2PO_4, 0.5% bovine serum albumin (defatted as in [7]). Mitochondrial protein was 0.6–0.8 mg in 1.5 ml total vol. The final concentrations of other added components are indicated in the results.

For calorimetric studies, the mitochondria were incubated in the same medium at 25°C. For technical reasons only a few experiments could be done at 37°C.

Heat output was recorded with an Arion differential microcalorimeter modified by addition of several microsyringes for direct injection into vessels. The apparatus had been calibrated by recording calorimetric responses after application of known currents through an internal reference resistance. The volume of the 2 vessels was 10 ml; each vessel contained 5 ml buffer with 0.6 mg mitochondrial protein/ml. The content of the vessels was mixed by rotating blades (60 rev./min).

3. Results and discussion

3.1. *Microcalorimetry of isolated mitochondria*

An example of the calorimetric response of brown fat mitochondria to addition of palmitoylcarnitine (PC) is shown in fig.1. The time course of this response was very similar to results with isolated cells stimulated by norepinephrime [5]. The maximal heat output was in the order of 0.7 mW/mg mitochondrial protein (fig.1, table 1). Parallel polarographic and calorimetric experiments have been done with different substrates and with brown adipose

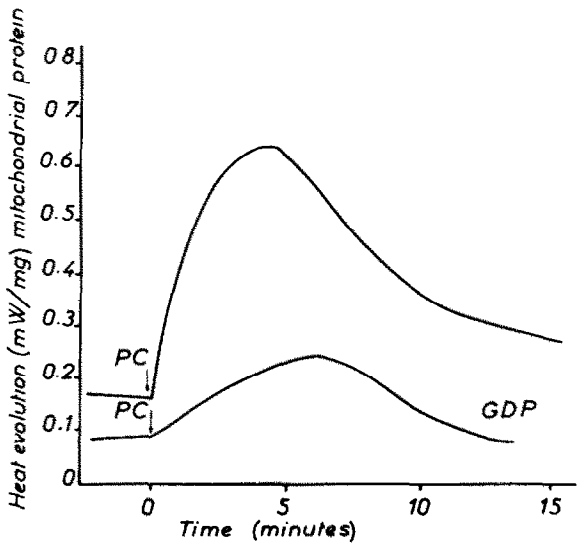

Fig.1. Calorimetric response of isolated brown adipose tissue mitochondria from cold-exposed rats. Effects of guanosine-diphosphate (GDP). Mitochondria were incubated in a medium as in section 2. The caloric output following addition of palmitoylcarnitine (PC 50 μM in the presence of malate 3 mM) was recorded with a differential microcalorimeter. Upper trace, no added GDP; Lower trace, GDP (1 mM) was added to mitochondria prior addition of PC.

tissue from control or cold-adapted animals (table 1). The maximum heat production was obtained with palmitoylcarnitine which is the physiological substrate. Under similar conditions, the heat output from liver mitochondrial was small (table 1), confirming the studies in [9]. The heat output values expressed per mg mitochondrial protein (0.6–0.8 mW) were very close to data in [5] with hamsters; the difference between rats and hamsters being that there is 5-times more total mitochondrial protein in the brown fat of hamsters.

When theoretical heat production was calculated from oxygen consumption a very good correlation was found between these data and heat production measured directly by microcalorimetry. These results lead to the conclusion that oxygen consumption can be used to estimate heat production by mitochondria. The same conclusion was obtained [5] using isolated cells. These data reinforce the observations that the apparent change in enthalpy is in agreement with the heat of combustion of substrates [8,9].

3.2. *The effect of GDP upon heat output from brown fat mitochondria*

The effect of added purine nucleotides on the decrease of respiration and the recoupling of brown fat mitochondria has been well documented [10–14]. The lower trace in fig.1 gives direct evidence that added GDP (before substrate addition) strongly reduced the heat production by mitochondria by restricting the energy dissipation. In brown fat mitochondria from cold-adapted rats, a striking increase of a 32 000 mol. wt membrane polypeptide has been observed [15,16]. This polypeptide has been shown to be the binding site of purine nucleotides [17].

Table 1
Comparison of theoretical and experimental heat production by brown fat mitochondria

	Substrate	O_2 consumption (nM. min.$^{-1}$. mg prot.$^{-1}$)	Theoretical heat production (mW. mg prot.$^{-1}$)	Experimental heat production (mW. mg prot.$^{-1}$)
Cold-exposed	PC	105	0.79	0.76
Control rats	PC	90	0.69	0.63
Cold-exposed rats	2 oxo-Glu	41	0.32	0.42
Cold-exposed rats	Succinate	62	0.46	0.50
Rat liver	PC	–	–	0.09

All data are expressed per mg mitochondrial protein and correspond to uncoupled respiration (no GDP) for brown fat mitochondria. Theoretical heat production was calculated from O_2 consumption (measured polarographically) with a caloric equivalent of 4.8 cal./ml O_2 (i.e., 421 μJ/nM O_2). (PC) palmitoylcarnitine (50 μM) in presence of malate (3 mM). (2 oxo-Glu) 2 oxo-glutarate (2 mM). With this substrate in presence of GDP the experimental heat output was 0.19 mW. Succinate (10 mM) in presence of rotenone (5 μM). All experiments were done at 25°C.

Table 2
Estimated heat production from total brown adipose tissue of 100 g rats

	Control rats	Cold-exposed rats
Heat production[a] microcalorimetry (mW. mg protein^{-1})	0.63	0.76
Heat production[b] for total brown adipose tissue (mW)	7	37
Tissue metabolism[c] (cal/h 25°C)	6	32
(cal/h 37°C)	15	80

[a] Considering that there is 600 mg total brown adipose tissue corresponding to 48 mg mitochondrial protein in cold-exposed rats and 250 mg tissue corresponding to 10 mg mitochondrial protein in control rats [6]
[b] was calculated from
[c] all experiments were done at 25°C. In order to convert the caloric output at 25°C in the estimated caloric output at 37°C a factor of 2.5 has been used, obtained from other experiments performed at 37°C

When binding sites are occupied by nucleotides, the H^+ (or OH^-) conductor is inhibited causing the H^+ electrochemical gradient to increase across the inner membrane. This increase induces respiratory control and inhibits heat production as predicted by the Mitchell theory [18].

3.3. *The estimated contribution of brown fat to non-shivering thermogenesis in rat*

The heat output from the tissue can be estimated from the data obtained from mitochondria (table 2). When considering that the maximal increase of total metabolism in a 100 mg cold-exposed rat is 1200 cal/h [19,23], it can be calculated that the contribution of brown fat to the increased cold-induced thermogenesis is ~7%. As emphasized [23,24], it seems important to consider in these conditions not the total heat production from brown fat but the special distribution of this heat to selected regions of the body. These results agree with data obtained with other methods [23–25,26]. However, strong discrepancies exist among data concerning the contribution of brown adipose tissue to non-shivering thermogenesis in rats [1,2,3,27].

Acknowledgements

The authors are indebted to Dr B. Cannon and Professor Ph. Hemon for valuable suggestions; the skillfull technical assistance of Christine Gervais is gratefully acknowledged. This work was supported in part by the Delegation à la Recherche Scientifique et Technique (contrat no. 77-77-0258).

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