ENERGETICS OF INVERTEBRATE ANOXIBIOSIS: DIRECT CALORIMETRY IN AQUATIC OLIGOCHAETES

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1. Introduction

Biochemical analyses of metabolic functions give insight into specific mechanisms of energy transformation in living organisms. In contrast, the biophysical approach by direct calorimetry reveals the sum of all the enthalpy changes associated with physico-chemical processes occuring within a defined system. Therefore the calorimetric method is particularly well suited for studying such complex phenomena as transitions between the aerobic and anoxic states of energy metabolism in invertebrates.

I have made high-resolution calorimetric measurements on the oligochaete *Lumbriculus variegatus*. Anoxic heat production is reduced by 40–80%, relative to aerobic rates. Interexperimental differences in anoxic heat production indicate different steady state levels of activity during anoxia. Enthalpy changes as calculated from biochemical data [1,2] explain <50% of the observed anoxic rates of heat production. These results point to deficiencies in our present knowledge of metabolic chemistry.

2. Experimental

In long-term calorimetric experiments controlled environmental conditions were maintained up to 7 days (av. 62 h). Water of known temperature, equilibrated with air (aerobic) or pure nitrogen (anoxic) was pumped at a constant 3.3 ml/h flowrate through the 0.5 ml pyrex chamber of a LKB-2107 flow sorption microcalorimeter. Gold capillary tubes prevented gaseous diffusion and minimized bacterial growth [3].

Oligochaetes were acclimated in aerated water at 16 ± 1°C for 3–20 days without addition of food. One size class of *Lumbriculus variegatus* was sorted out, cleaned, and weighed. Interindividual variability in weight was < ± 20%. In the experimental chamber without substrate the animals formed a cluster and resumed their typical 'searching movements'. Mechanically induced and spontaneous bursts of swimming activity, involving the whole group, were observed before insertion of the chamber into the calorimeter.

3. Results

3.1. *Anoxic and aerobic heat production*

Under anoxic conditions a steady rate of heat production was obtained, whereas under aerobic conditions the animals often showed synchronized peaks of activity (fig.1). Maintenance of a constant level of heat production during prolonged anoxic exposures (up to 48 h) indicates the establishment of a dynamic equilibrium under these conditions which was not altered by addition of 8% CO_2 to the nitrogen. When the animals were returned to aerobic conditions, heat production increased sharply, and an overshoot (analogous to an 'oxygen debt') was frequently observed (fig.1).

The ratio of anoxic to aerobic heat production increases with increasing levels of metabolism at a given temperature (fig.2).

3.2. *Effect of temperature and state of activity*

At any one temperature, the rates of metabolism of different groups of animals varied considerably, and anoxic and aerobic rates of heat production were correlated (fig.2, inset). This indicates different levels of metabolic activity of the animals, the relative
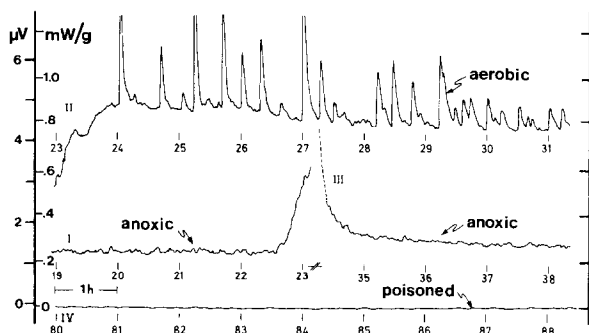
Fig.1. Superimposed segments of an experiment showing aerobic and anoxic rates of heat production of a group of 10 *Lumbriculus variegatus* (120.4 mg wet wt) at 20°C. The time (in hours) from the beginning of the experiment is indicated beneath each segment; roman numerals identify the segments of the record, as follows: I, a constant anoxic rate of heat production from 14–22.5 h (19–22.5 h shown); II an aerobic overshoot (from 23–~28 h) with peaks of high activity, followed by steady-state aerobic heat production from 28–34 h (28–31 shown); III, equilibration with anoxic water (34–36 h), followed by a stable anoxic rate from 36–44 h (36–38 h shown); IV, after poisoning the animals (Amoquar, Pfizer Corp.; 1 μl/ml), a smooth baseline (80–95 h; 80–88 h shown) indicates that the system is relatively free of noise and drift [3]. A constant voltage above the baseline is directly proportional to the specific rate of heat production (mW/g) calculated from the electrically determined calibration constant (54.7 ± 0.87 μV/mW) and the biomass. Anoxic rate of heat production is 31% of the aerobic rate, as determined by integrating and averaging the aerobic and anoxic segments of the thermogram, omitting periods of equilibration and overshoot.

variability of anoxic heat production amounting to twice that of the aerobic rates (table 1). This wide range of the 'scope of anoxic activity' reflects a high metabolic flexibility which may be characteristic of animals which can survive persistent anoxia.

The lowest measured rates are virtually independent of temperature, as has been ascribed to the standard rate of metabolism of some animals [4]. This reduction in temperature dependence of the standard rate as well as the reduced energy costs of maintenance may constitute decisive features of anoxic tolerance.

4. Discussion

Discrepancy between direct and indirect calorimetry of anoxibiosis

To what extent do presently known biochemical reactions explain the observed heat production? Bio-

energetic investigations of aerobic heat production have usually revealed satisfactory agreement between direct calorimetric and indirect (respirometric) methods [5–8] (for a possible exception see [9]). Furthermore, there is no reason to assume that anoxic
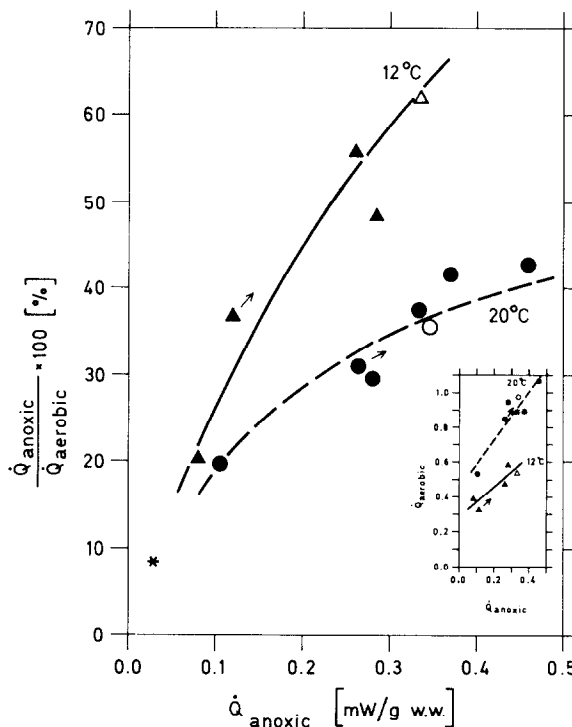

Fig.2. Differential temperature–activity effect on the relation between anoxic and aerobic rate of heat production of *Lumbriculus variegatus*. Closed symbols: specific rates of heat production averaged over 3–65 h aerobic and anoxic steady state levels in different experiments each with 10 animals (10.3 mg wet wt/individual $\pm$ 1.1 SD). The linear relationship between anoxic and aerobic specific rates of heat production (inset):

$$\dot{Q}_{aerobic} = (a + b) \times \dot{Q}_{anoxic}$$

results in a hyperbolic function between the anoxic/aerobic ratio (R) and anoxic rate of heat production ($\dot{Q}_{anoxic}$):

$$\frac{1}{R} = (b + a) \times \frac{1}{\dot{Q}_{anoxic}}$$

Open symbols: stimulating effect of antibiotics (streptomycin- and neomycin-sulfate, 200 μg/ml at 12°C; 20 μg/ml at 20°C) on the rate of heat production in experiments indicated by arrows. Note that with increasing levels of metabolic activity the effects of temperature become more pronounced.

Table 1

Comparison of measured heat production of *Lumbriculus variegatus* ($\dot{Q}$) with calculated heat production of Tubificidae from oxygen uptake (O_2) and biochemical endproduct determinations (p)

Conditions	Heat production (mW/g)	
	12°C	20°C
Aerobic ($\dot{Q}$)	0.44 ± 0.11 (4)	0.86 ± 0.18 (6)
Aerobic (O_2)[a]	0.55 ± 0.16 (3)	0.89 ± 0.30 (4)
$O_2/\dot{Q} \times 100$[c]	125% (71–215%)	103% (57 to 175%)
Anoxic ($\dot{Q}$)	0.19 ± 0.10 (4)	0.30 ± 0.12 (6)
Anoxic (p)[b]	0.065 ± 0.004 (3)	
$p/\dot{Q} \times 100$[c]	27% (17 to 52%)	

Comparison of *Lumbriculus* and *Tubifex* is valid, because the two species exhibit essentially the same rates of oxygen uptake [19]. Numbers are means ± SD with the number of determinations or number of reference values in brackets

[a] Calculated from respiration rates [20–24] obtained by use of polarographic oxygen sensors or Winkler analysis. The manometric methods involve shaking of the apparatus resulting in up to 2-fold increases of respiratory activity and have therefore not been used [25]. –450 kJ/mol O_2 is used as caloric equivalent of oxygen, as mainly fat and some protein is metabolized in aerobically starved Tubificidae [2,20]

[b] Calculated from biochemical endproduct determinations [2] at about 16°C (Schöttler, personal communication) omitting the first hours of anoxia when metabolic equilibrium is not yet reached. Stoichiometric equations of glycogen fermentations [1] provide the basis for estimating the enthalpy changes of the reactions in vivo. These were calculated from heats of formation of dissolved substances at 25°C, infinite dilution in a hypothetical buffer solution at pH 7, and a heat of neutralization of –30 kJ/mol H^+ [10]. These conditions are chosen to provide maximal estimations of the heat effects accompanying fermentative reactions under physiological conditions. Accordingly the maximal caloric equivalent of the average anoxic endproduct of *Tubifex* (biochemical data in [2]) is – 146 kJ/mol organic acid. Dry weight is 17% of the wet weight

[c] Percentage of calculated to measured rate of heat production; mean values with limits of ratios given in brackets as $(\overline{x}_1 - s_1)/(\overline{x}_2 + s_2) \times 100$ to $(\overline{x}_1 + s_1)/(\overline{x}_2 - s_2) \times 100$. Aerobic rates are not significantly different, while the unexplained fraction of anoxic heat production is highly significant

reactions contribute significantly to aerobic heat production of *Lumbriculus*, since pure oxygen (for 8 h) did not increase the aerobic rate. Measured and calculated values of aerobic rates of heat production are not significantly different (table 1).

However, if anoxic heat production as measured in the calorimeter is compared with enthalpy changes as calculated for fermentative reactions under physiological conditions [10] a large discrepancy emerges (table 1). Biochemical studies with oligochaetes show that anoxic acclimation favors the formation of secondary endproducts of glycogen fermentation (propionate, acetate) [2,11,12] with high yields of ATP [1]. However, <50% of the heat production measured under anoxia can be explained by these biochemical reactions. Thermodynamic analysis of the metabolic chemistry of bivalves (*Mytilus edulis*) [13,14] as compared to direct calorimetric data [15] reveals similar differences (E. G., unpublished). Energy balance studies of vertebrate muscles [16,17] and of human erythrocytes [18] have also shown that a large fraction of anoxic heat production remains unaccounted for. The search for the sources of these unexplained flows of heat from tissues under anoxia presents a challenge to the comparative physiologist as well as to the biochemist.

Acknowledgements

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