Journal of Thermal Biology 69 (2017) 267–274

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Aerobic function in mitochondria persists beyond death by heat stress in insects

Erica C. Heinrich[a,*], Emilie M. Gray[b], Ashley Ossher[a], Stephen Meigher[b], Felix Grun[c], Timothy J. Bradley[a]

[a] Department of Ecology and Evolutionary Biology, University of California, Irvine, 321 Steinhaus Hall, Irvine, CA 92697-2525, USA
[b] Department of Organismal Biology & Ecology, Colorado College, 14 East Cache La Poudre St., Colorado Springs, CO 80903, USA
[c] Center for Complex Biological Systems, University of California, Irvine, 2620 Biological Sciences III, Irvine, CA 92697-2280, USA

ARTICLE INFO

Keywords:
Thermal maximum
Insect
Respiration
Tracheal system
Mitochondria

ABSTRACT

The critical thermal maximum (CT_{max}) of insects can be determined using flow-through thermolimit respirometry. It has been demonstrated that respiratory patterns cease and insects do not recover once the CT_{max} temperature has been reached. However, if high temperatures are maintained following the CT_{max}, researchers have observed a curious phenomenon whereby the insect body releases a large burst of carbon dioxide at a rate and magnitude that often exceed that of the live insect. This carbon dioxide release has been termed the post-mortal peak (PMP). We demonstrate here that the PMP is observed only at high temperatures, is oxygen-dependent, is prevented by cyanide exposure, and is associated with concomitant consumption of oxygen. We conclude that the PMP derives from highly active, aerobic metabolism in the mitochondria. The insect tracheal system contains air-filled tubes that reach deep into the tissues and allow mitochondria access to oxygen even upon organismal death. This unique condition permits the investigation of mitochondrial function during thermal failure in a manner that cannot be achieved using vertebrate organisms or *in vitro* preparations.

1. Introduction

Thermal tolerance is a major determinant of individual survival, population distribution (Angiletta, 2009; Blackburn et al., 2014; Hoffmann, 2010; Huey and Kingsolver, 1993; Somero, 2010), and predicting a species' fate in the context of climate change (Deutsch et al., 2008). The study of extreme temperature tolerance has received a great deal of attention in insects, and multiple methods have been developed to compare thermal limits among individuals and populations (Terblanche et al., 2011). While these methods have provided valuable data on whole organism thermal limits, studies rarely examine the specific cellular and sub-cellular processes that lead to metabolic failure.

A number of hypotheses may explain the mechanistic basis of thermal death. One possible cause is protein destabilization at high temperatures (Hochachka and Somero, 2002), leading to reduction and cessation of enzymatic activity. Another possible cause of physiological failure at high temperatures involves temperature-induced increases in the fluidity of membrane lipids (Cooper et al., 2014; Grim et al., 2010). Lipid phase changes at high temperature can affect protein movement and aggregation, as well as membrane permeability. It has also been argued that death at high temperatures can be due to metabolic mismatch. For example, Pörtner (2001) has suggested that the rate of oxygen delivery becomes insufficient at high temperature to meet the increasing demands of aerobic metabolism. In aquatic invertebrates, he demonstrated that high temperature leads to oxygen starvation and death. Klok et al. (2004), however have suggested that this explanation may not apply to terrestrial animals. In contrast to water, oxygen concentration in air is not significantly affected by temperature. Additionally, the insect tracheal system is extremely effective in transporting oxygen and carbon dioxide as a result of their increased diffusion coefficients in air *versus* water (Krogh, 1920a, 1920b; Weis-Fogh, 1964; Wigglesworth and Lee, 1982).

Thermolimit respirometry, a technique developed by Lighton and Turner (2004) to determine the critical thermal maximum (CT_{max}), may help us investigate the mechanistic basis of thermal death. This method involves measuring gas exchange (both the volume and pattern of CO_2 production) using flow-through respirometry as temperature is ramped

Abbreviations: PMP, post-mortal peak; CT_{max}, critical thermal maximum

* Corresponding author. Present address: Division of Physiology, Department of Medicine, University of California, San Diego, 9500 Gilman Drive, 0623A, La Jolla, CA 92093-0623, USA.
E-mail address: echeinrich@ucsd.edu (E.C. Heinrich).

http://dx.doi.org/10.1016/j.jtherbio.2017.08.009
Received 26 July 2016; Received in revised form 7 August 2017; Accepted 17 August 2017
Available online 24 August 2017

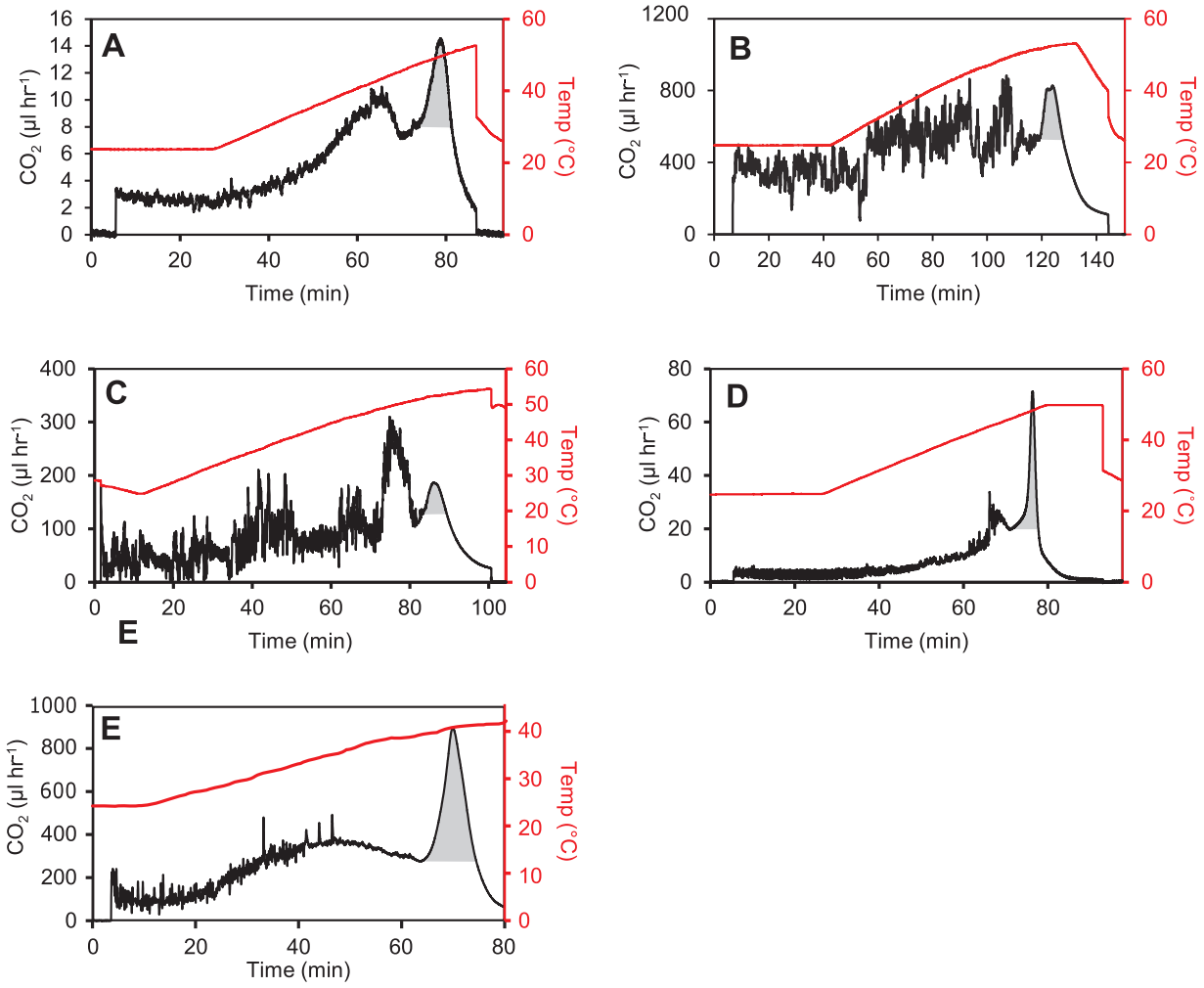

Fig. 1. The prevalence of the PMP. CO_2 release over time during a thermal ramp. Temperature of the airstream is indicated in red. The PMP volume measured is highlighted in grey from its approximate start to end points. Data represent the following species: (A) *Drosophila melanogaster*; (B) *Acheta domesticus*; (C) *Gromphadorhina portentosa*; (D) *Culex pipiens*; (E) *Tenebrio molitor*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

up from ambient to lethal values. In this method, the CT_{max} is identified as the point at which spiracular control is lost due to failed muscular coordination (Miller, 1964). The insect metabolic rate increases with temperature but then declines at temperatures just below the CT_{max}, demonstrating the gradual, pre-mortal temperature-dependent failure of the metabolic pathways. Vorhees and Bradley have demonstrated that insects are functionally dead at the CT_{max} temperature (2012). It is surprising therefore that, if the temperature is increased beyond the CT_{max}, a large burst of CO_2 is released. The rate of CO_2 release during this post mortal peak (PMP) is, in some species, greater than the highest metabolic rate observed in the live insect.

The post-mortal peak has to date only been demonstrated in insects. It is even absent in small arthropods such as pill bugs that lack tracheae (Klok et al., 2004; Schmidt and Wägele, 2001). When insects die, their spiracles open due to the failure of the spiracular closer muscles. As a result, the tracheae provide an open diffusive pathway leading from the atmosphere to the deepest tissues. We are therefore able to observe and measure metabolic changes in the tissues occurring at high temperatures immediately prior to, and after, death. Such insights are not possible with vertebrates where death leads to the immediate cessation of gas transport to and from the exterior.

While the PMP has been repeatedly observed during thermolimit respirometry (Folk et al., 2007; Klok et al., 2004; Lighton and Turner, 2004; Lighton, 2007; Stabentheiner et al., 2012; Stevens et al., 2010; Vorhees et al., 2013), its source remains unknown. Only one earlier study (Mölich et al., 2012) has explored this phenomenon and demonstrated that the PMP in *Drosophila melanogaster* occurs only in the presence of oxygen. We propose three hypotheses to explain CO_2 release during the PMP: 1) it represents CO_2 stored in the hemolymph or tissues that is released post-mortem at high temperatures, 2) the CO_2 derives from microbial metabolism in the insect's body, or 3) the CO_2 derives from insect tissue metabolism, albeit at temperatures that exceed CT_{max}. The present study tests these hypotheses in an attempt to understand the timing of cellular events before and after thermal death.

The PMP has been observed in a number of insect species (Folk et al., 2007; Klok et al., 2004; Lighton and Turner, 2004; Lighton, 2007; Stabentheiner et al., 2012; Stevens et al., 2010; Vorhees et al., 2013; Vorhees and Bradley, 2012). We examined the PMP in 5 insect species belonging to 4 orders (Diptera, Coleoptera, Orthoptera, Blattodea). Our goal was to examine a diverse set of species to test for the phylogenetic breadth of our observations. Our results provide new insights into the physiology of thermal tolerance and the relative roles of the eukaryotic cellular machinery and mitochondria in this process.

2. Materials and methods

Thermolimit respirometry procedures were modified from Lighton and Turner (2004). In all thermolimit trials, the following experimental setups were used. Air was pushed by either a hydrostatic pump (Sable Systems International, Las Vegas, NV, USA) or a pressurized lab air