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Mitochondrial phenotype during torpor: Modulation of mitochondrial electron transport system in the Chilean mouse–opossum *Thylamys elegans*

Pablo A. Cortes[a,b,*], Francisco Bozinovic[b], Pierre U. Blier[c]

[a] Escuela de Agronomía, Facultad de Ciencias, Universidad Mayor, Camino La Pirámide, 5750, Huechuraba, Chile
[b] Departamento de Ecología, Center of Applied Ecology and Sustainability, Facultad de Ciencias Biológicas, Universidad Católica de Chile, Santiago 6513677, Chile
[c] Département de Biologie, Laboratoire de Physiologie Animale Intégrative, Université du Québec, Rimouski G5L 3A1, QC, Canada

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ABSTRACT

Mammalian torpor is a phenotype characterized by a controlled decline of metabolic rate, generally followed by a reduction in body temperature. During arousal from torpor, both metabolic rate and body temperature rapidly returns to resting levels. Metabolic rate reduction experienced by torpid animals is triggered by active suppression of mitochondrial respiration, which is rapidly reversed during rewarming process. In this study, we analyzed the changes in the maximal activity of key enzymes related to electron transport system (complexes I, III and IV) in six tissues of torpid, arousing and euthermic Chilean mouse-opossums (*Thylamys elegans*). We observed higher maximal activities of complexes I and IV during torpor in brain, heart and liver, the most metabolically active organs in mammals. On the contrary, higher enzymatic activities of complexes III were observed during torpor in kidneys and lungs. Moreover, skeletal muscle was the only tissue without significant differences among stages in all complexes evaluated, suggesting no modulation of oxidative capacities of electron transport system components in this thermogenic tissue. In overall, our data suggest that complexes I and IV activity plays a major role in initiation and maintenance of metabolic suppression during torpor in Chilean mouse–opossum, whereas improvement of oxidative capacities in complex III might be critical to sustain metabolic machinery in organs that remains metabolically active during torpor.

1. Introduction

An evolutionary convergent physiological state displayed by all major animal phyla to regulate their energy budgets is dormancy (i.e. metabolic depression; Guppy and Withers, 1999). In small endotherms, metabolic depression associated with deep (hibernation) and shallow (daily) torpor is a well-studied example (Bozinovic et al., 2004; Geiser, 2004; Melvin and Andrews, 2009; Ruf and Geiser, 2015). Specifically, many small mammalian species exhibit torpor to cope with the energetic constraints. The environmental cues triggering daily, shallow torpor seem to be a combination of three factors: reduced food availability/quality, decreased ambient temperature and shortened photoperiod (Geiser, 1994; Bozinovic and Méndez, 1997; Bozinovic et al., 2007; Turner and Geiser, 2017). During torpor, animals experience a decrease in metabolic rate below resting values, generally followed by a reduction in body temperature. In therian mammals (i.e. marsupials and placental mammals) daily and seasonal (i.e. hibernation) torpor are two distinct phenotypes, which differ in the degree of hypometabolism and hypothermia experienced during torpor bouts (Geiser and Ruff, 1995; Wilz and Heldmaier, 2000; Lovegrove et al., 2001; Nespolo et al., 2010). During winter, torpid and hibernating animals experiences repeatedly torpor bouts, each including three phases: deep torpor, arousal and post-torpor normothermy (euthermy). The torpor phase is characterized by a complete suppression of almost all metabolically active physiological processes allowing energy saving. Nevertheless, some processes must operate at lower levels of activity, since they are critical for survival (Carey et al., 2003; Staples and Brown, 2008).

Arousal from torpor is a critical episode, where whole-animal metabolic rate and body temperature rapidly returns to typical normothermic levels, passing through a peak of metabolic activity. In fact, during this short period, heart rate might change from five to 400 beats/min, body temperature increases by > 30 °C and metabolic rate increases by 500-fold (Fons et al., 1997; Milsom et al., 1999; Opazo et al., 1999; Hampton et al., 2010). This rapid increase in temperature and tissue reperfusion can provoke extensive pathophysiological conditions leading to tissue necrosis in absence of a precise coordination of the rewarming process, oxygen supply and metabolic activation (Bradbury, 2001). Many tissues and organs are not re-activated at the same rate during arousal. Several studies have shown that heart and liver rewarm much faster than others tissues and have a key role in

* Corresponding author at: Escuela de Agronomía, Facultad de Ciencias, Universidad Mayor, Camino La Pirámide, 5750, Huechuraba, Chile.
E-mail address: pablocortesgarcia@gmail.com (P.A. Cortes).

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physiological reactivation during arousal (Osborne et al., 2005; Hampton et al., 2010). According to Hampton et al. (2010), rodent's arousal from torpor is limited by the capacities of the cardiovascular system, which start to perfuse the most metabolically active organs (heart, brain, liver and brown adipose tissue) in a specific and "programmed" progression. During rewarming the first line of metabolic increase involves the heart and lungs, which not only reactivate blood flow through the thorax and the liver (Hirshfeld and O'Farrell, 1976; Hampton et al., 2010) but also increase their specific metabolism several times over their normothermic values (Andrews et al., 2009; Hampton et al., 2010). Concomitant with this metabolic elevation, an increase in filtration rate by kidneys also occurs, increasing in turn their specific metabolism (Zatzman, 1984).

Metabolic rate reduction during torpor is triggered by active suppression of mitochondrial respiration, which can explain from 30% up to 70% of metabolic rate reduction during entrance to torpor in daily torpid and hibernating mammals, respectively (Guppy and Withers, 1999; Brown and Staples, 2010; Staples, 2014; but see Grimpo et al., 2014). Most critical, during rewarming process this suppression is rapidly reversed. Common to both physiological states there is a concomitant regulation of the electron transport system (ETS) complexes. For example, a decrease in oxidative capacities of NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) in liver and skeletal muscle mitochondria has been documented in hibernating squirrels. Additionally, complex II activity typically increases during arousal (Armstrong and Staples, 2010; Chung et al., 2011; Brown et al., 2013). However, inhibition of complex I and II does not account for all the metabolic suppression observed during daily torpor and hibernation (Armstrong and Staples, 2010). In general, the role of ETS regulation is controversial, as some authors have reported absence of regulation and up-regulation depending on species and tissues (Blank et al., 1988; Brustovetsky et al., 1990; Eddy et al., 2006; Kutschke et al., 2013). In fact, Kutschke et al. (2013) proposed that mitochondrial modulations during daily torpor are mainly specific to the liver.

To the best of our knowledge, studies examining modulation of mitochondrial aerobic metabolism along the torpor-arousal cycle have been limited exclusively to placental mammals. It is well known that marsupials and placental mammals differ in many characters (Renfree, 1981; Geiser and Ruff, 1995; Nicol et al., 1997). One major difference between both groups is in the thermoregulatory mechanism used during rewarming. In placental mammals, the major mechanism of heat production during rewarming is non-shivering thermogenesis (NST) (Morrison et al., 2008; Nowack et al., 2013). Heat production by NST is the consequence of fatty acid oxidation and uncoupling of oxidative phosphorylation in brown adipose tissue (BAT) (Cannon and Nedergaard, 2004). A BAT-specific protein, the uncoupling protein 1 is responsible to dissipate the proton gradient formed across the inner mitochondrial, preventing the synthesis of ATP and transforming energy into heat (Matthias et al., 2000; Nedergaard et al., 2001). In marsupials, unlike placental mammals, the capacity for BAT-dependent NST and its relative importance in rewarming is still a matter of active debate (Kabat et al., 2003; May, 2003; Rose and Ikonomopoulou, 2005; Cortés et al., 2014). In these mammals, however, shivering thermogenesis (ST) is the main mechanisms associated with arousal from torpor, which is consequence of rapid and isometric muscle contractions (Banet et al., 1978; Cotton, 2016).

In the present study, we characterized activities of mitochondrial ETS components associated to different states of torpor-arousal cycle in the marsupial, *Thylamys elegans* or the Chilean mouse-opossum (Silva-Duran and Bozinovic, 1999; Bozinovic et al., 2005; Cortés et al., 2016). Specifically, we compared the maximal activity of complex I, III and IV in six organs of torpid, arousing and euthermic mouse-opossum. Since metabolic rate reduction during torpor could be triggered by an ETS inhibition, we expected lower enzymatic activities of ETS components in torpid animals. Furthermore, as different studies have shown that heart and liver rewarm much faster than others tissues (Hampton et al., 2010), we then expected that mitochondrial oxidative capacities and the different components of ETS should be modulated during rewarming in liver and heart to cover coordinated reactivation of mitochondrial function. This coordinated reactivation is suspected to be required to insure, among others, better control of ETS reduction state as well as oxidative stress that could result from rapid re-oxygenation of the tissues. In a previous study, Opazo et al. (1999) reported the absence of NST in *T. elegans*, suggesting that ST is the main thermoregulatory mechanisms during rewarming process in this marsupial species. Thus, we expected higher enzymatic activities of ETS components in skeletal muscle from arousing Chilean mouse–opossum.

2. Material and methods

2.1. Animals

Sixteen adult individuals of *T. elegans* were captured in Central Chile (33° 29′S; 70° 56′W) during the Austral Autumn with Sherman traps located in the ground and bated with bananas and fish. Individuals were transported to the laboratory immediately after capture. Animals were individualized and housed in plastic cages ($45 \times 30 \times 20\ cm^3$) with 2 cm of bedding. All individuals were maintained for two weeks in a temperature-controlled room at 20 ± 1 °C with a 12:12 photoperiod. Food (mealworms and ground beef) and water were provided ad libitum.

2.2. Torpor induction and experimental design

Torpor induction was carried out following procedure previously reported by Cortés et al. (2016). The first step was the weighing of all active animals and subsequently euthermic body temperature ($T_{b\ NOR}$) was measured using a copper-constantan thermocouple inserted into the cloaca. Then, animals were transferred to a temperature-controlled chamber (Pitec Instrument, Chile) at 5 ± 1 °C with a 12:12 photoperiod. Animals were visually monitored each one-hour to determine whether they were torpid or active. When an individual became torpid, we waited for approximately 12 h to take a new body mass and body temperature measurement ($T_{b\ TOR}$). Five torpid animals were sacrificed immediately after $T_{b\ TOR}$ measurements by a rapid decapitation. Non-sacrificed animals were transferred to a temperature-controlled room (20 ± 1 °C) and copper-constantan thermocouple were gently inserted into the cloacae and attached to the tail with cloth tape. This manipulation was enough to induce arousal (Opazo et al., 1999). Seven arousing animals were sacrificed after they reached middle point between normothermic and torpid body temperature ($T_{b\ NOR} - T_{b\ TOR}/2$). Four post-torpor normothermic animals were sacrificed after reaching their initial normothermic body temperature ($T_{b\ EUT}$).

2.3. Tissue collection and enzymatic assays

Brain, heart, kidneys, liver, lung and skeletal muscle were collected after euthanasia and immediately frozen in liquid nitrogen and maintained at −80 °C. Samples were shipped to Université du Québec à Rimouski (Canada) on dry shipper at −80 °C. Each tissue was homogenized in 19 volumes of homogenization solution (10 mM HEPES, 50 mM potassium phosphate and 0.5 mM EDTA; Ph 7.4) using three 10 s bursts with a Tissue Tearor homogenizer (Biospec Products Inc., Bartlesville, OK, USA). Enzyme activity was measured at 24 °C using an Envision Multilabel Plate Reader (Perkin Elmer, Waltham, MA) spectrophotometer. Enzyme activities were measured at only one temperature because our objective was to compare maximal activities in different tissues and conditions (i.e., torpor, arousal, euthermy) to detect up or down modulation of activities. In these conditions, maximal activity is a proxy of both the content and catalytic capacity of the different enzymes. All assays were run in triplicate and specific activities were expressed as $\mu mol\ min^{-1}\ mg^{-1}$ protein (Bradford protein-dye