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Torpor-arousal cycles in Syrian hamster heart are associated with transient activation of the protein quality control system

Marit Wiersma[a,b,*], Thais M.A. Beuren[a,b], Edwin L. de Vrij[b,c], Vera A. Reitsema[b], Jantje J. Bruintjes[b], Hjalmar R. Bouma[b,d], Bianca J.J.M. Brundel[a,b], Robert H. Henning[b]

[a] Department of Physiology, Amsterdam Cardiovascular Sciences, VU University Medical Center, Amsterdam, The Netherlands
[b] Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, The Netherlands
[c] Department of Surgery, Martini Hospital, Groningen, The Netherlands
[d] Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

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ABSTRACT

Hibernation consists of torpor, with marked suppression of metabolism and physiological functions, alternated with arousal periods featuring their full restoration. The heart is particularly challenged, exemplified by its rate reduction from 400 to 5–10 beats per minute during torpor in Syrian hamsters. In addition, during arousals, the heart needs to accommodate the very rapid return to normal function, which lead to our hypothesis that cardiac function during hibernation is supported by maintenance of protein homeostasis through adaptations in the protein quality control (PQC) system. Hereto, we examined autophagy, the endoplasmic reticulum (ER) unfolded protein (UPR_{ER}) response and the heat shock response (HSR) in Syrian hamster hearts during torpor and arousal. Transition from torpor to arousal (1.5 h) was associated with stimulation of the PQC system during early arousal, demonstrated by induction of autophagosomes, as shown by an increase in LC3B-II protein abundance, likely related to the activation of the UPR_{ER} during late torpor in response to proteotoxic stress. The HSR was not activated during torpor or arousal. Our results demonstrate activation of the cardiac PQC system – particularly autophagosomal degradation – in early arousal in response to cardiac stress, to clear excess aberrant or damaged proteins, being gradually formed during the torpor bout and/or the rapid increase in heart rate during the transition from torpor to arousal. This mechanism may enable the large gain in cardiac function during the transition from torpor to arousal, which may hold promise to further understand 'hibernation' of cardiomyocytes in human heart disease.

1. Introduction

Hibernation allows mammals to survive challenging environmental conditions, such as low temperatures and food shortage, by limiting energy expenditure through lowering of metabolism during periods of torpor. Torpor phases, lasting from several days to weeks, are alternated with much shorter arousal periods (interbout arousal), during which metabolism is restored. During torpor, metabolic rate is suppressed > 95% of euthermic (summer) rates, resulting in a reduction of body temperature (T_b) from 37 °C to a few degrees above ambient temperature (T_a), often as low as 0–5 °C. In addition, key physiological functions undergo drastic changes during torpor, which are restored to levels even exceeding those observed in summer animals during arousal (Carey et al., 2003; Geiser, 2004; McArthur and Milsom, 1991; Zatzman, 1984).

The mammalian heart is particularly challenged during hibernation. During torpor, the heart needs to balance its suppressed metabolism with providing sufficient cardiac output in the face of a largely increased peripheral vascular resistance (Heinis et al., 2015), whilst precluding arrhythmias and cardiac arrest due to low T_b (Johansson, 1996). In addition, during interbout arousals, the heart needs to accommodate the very rapid return to normal function, which markedly exceeds baseline summer function because of low T_a, thermogenesis and shivering. Adaptations of cardiac physiology and morphology during torpor include a reduction of heart rate from 400 to 5–10 beats per minute, an increase in contractility and cardiac hypertrophy (Nelson and Rourke, 2013). Various adaptations in Ca^{2+} homeostasis ensure a low intracellular Ca^{2+} concentration, which confers resistance of torpid hearts to arrhythmias provoked by an intracellular Ca^{2+} overload (Fedorov et al., 2008; Giroud et al., 2013; Johansson, 1996; Li et al., 2011; Nakipova et al., 2017; Yatani et al., 2004). Molecular studies have documented cardiac expression changes mainly during

* Corresponding author at: Department of Physiology, De Boelelaan 1117, 1081HV Amsterdam, The Netherlands.
E-mail address: m.wiersma1@vumc.nl (M. Wiersma).

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preparation for hibernation to adapt metabolism towards beta-oxidation, harness the cardiomyocytes by deploying a foetal expression program (gene expression and molecular changes resembling those during foetal cardiac development, including the preference of carbohydrates over fatty acids as substrates for ATP generation in a hypoxic environment) and increase oxidant defence (Grabek et al., 2011; Heinis et al., 2015; Nelson and Rourke, 2013; Vermillion et al., 2015).

While pre-hibernation adaptations ensure proper heart function during torpor, the transitions between torpor and arousal may still require additional measures to cope with cardiac stress. We recently identified the need for a proper maintenance of proteostasis, i.e. the synthesis, folding, trafficking and degradation of proteins, to prevent cardiac dysfunction and arrhythmogenicity in models of acute cardiac stress induced by rapid pacing (Brundel et al., 2006; Meijering et al., 2012; Wiersma et al., 2017; Zhang et al., 2014). Patency of proteostasis is monitored by the protein quality control (PQC) system, which responds to cellular stress by mounting the heat shock and unfolded protein responses and by degrading aberrant proteins by the autophagic-lysosomal pathway (Meijering et al., 2015; Powers and Balch, 2013). Maintaining proteostasis is challenged in (early) arousal especially, as the associated rapid increase in cardiac activation rate and hemodynamic load likely induces cardiomyocyte stress, which may lead to imbalances in redox homeostasis and protein damage. In addition, the reduction in metabolism during torpor influences proteostasis, by inhibiting protein synthesis and changing protein stability, function and binding properties, partly conferred through modification of protein phosphorylation (Carey et al., 2003; Epperson et al., 2010a,b; Frerichs et al., 1998; Hindle et al., 2014; Storey, 1997; Whitten and Klain, 1968). Furthermore, proteomic studies in hibernating ground squirrels documented an induction of several stress-related proteins, including heat shock proteins, during late torpor or arousal in liver (Xu et al., 2013), brain (Epperson et al., 2010b) and skeletal muscle (Hindle et al., 2011), whereas heart has not been examined. Expression of GRP78/BiP, a protein involved in the endoplasmic reticulum (ER) unfolded protein response (UPR_{ER}), was found altered in several tissues, including heart, during torpor and arousal (Mamady and Storey, 2006).

Although changed activation of the PQC system has been found in hibernating mammals, these studies only document the differences of PQC activation between summer and torpid animals. Changes in the PQC system within the different hibernation phases (i.e. torpor and arousal) have never been examined. We hypothesized that the activity of the PQC system in heart tissue is reduced during torpor and increases during interbout arousal in the hibernating Syrian hamster. Hereto, we examined cardiac macroautophagy (further referred to as 'autophagy'), a lysosomal degradation pathway of damaged and/or aged proteins, macromolecules and organelles, by measuring the expression levels of LC3B-II, a protein correlating with the levels of autophagosomes (Kabeya et al., 2000) and phosphorylation of mTOR (Ser2448), a major switch controlling the activation of autophagy (Chou et al., 2012; Codogno and Meijer, 2005). Cardiac UPR_{ER} activation was measured by GRP78/BiP protein expression, phosphorylation of eIF2α and mRNA expression of ATF4 and ATG12. In addition, the heat shock response (HSR) was analysed by quantifying the expression of HSF1 and HSP25.

2. Material and methods

2.1. Ethical approval

Experiments were approved by the Institutional Animal Ethical Committee of the University Medical Center Groningen. Animal procedures were carried out in accordance with the European Directive on the Protection of Animals used for Scientific Purposes and Dutch legislation. Experiments were performed on Syrian hamsters (*Mesocricetus auratus*) from Envigo (Indianapolis, USA) with free access to food and water ad libitum. Euthanasia was performed by intraperitoneal injection of an overdose of pentobarbital (600 mg/kg), followed by exsanguination within 5 min.

Table 1
Body temperature and weight of Syrian hamsters at euthanization.

	n	T_b (°C)	T_m (°C)	Weight (g)
TE	10	5.8 ± 0.6	5.6 ± 0.6	109.2 ± 7.2
TL	16	6.5 ± 0.6	6.1 ± 0.4	102.8 ± 4.6
AE	15	$31.0 \pm 0.5^{TE,TL}$	$34.3 \pm 0.7^{TE,TL}$	90.9 ± 5.3
AL	14	$34.2 \pm 0.7^{TE,TL}$	$35.1 \pm 0.7^{TE,TL}$	101.9 ± 5.0

Values are presented as mean value ± SEM or number of animals (n). T_b: body temperature (core), T_m: temperature mouth. Significant differences ($P < .05$) between groups are indicated by the superscripts. (TE: early torpor, TL: late torpor, AE: early arousal, AL: late arousal.)

2.2. Animals

Male and female Syrian hamsters were housed at an ambient temperature of 21 °C and a light:dark-cycle (L:D-cycle) of 14:10 h. Hibernation was induced by shortening the L:D-cycle to 8:16 h for 10 weeks followed by housing at an ambient temperature of 5 °C at continuous dim light (< 5 Lux) (Bouma et al., 2011, 2013). The hibernation patterns of animals were determined by continuous assessment of movement via infrared detectors connected to a computer. Syrian hamsters with > 24 h of inactivity were considered torpid (Oklejewicz et al., 2001). Animals were euthanized during torpor or interbout arousal, the final duration of uninterrupted torpor bout lasted > 80 h for all animals. The animal's activity pattern accurately identified hibernating hamsters as being in torpor or arousal, as evidenced by mouth and core T_b at euthanization (Table 1). Early arousal was induced by handling of the animals, with animals being euthanized 90 min later. Movement was not used to asses arousal, but T_b measurement was. Hearts were removed; the lower 1/3 of the ventricles (mainly representing left ventricle free wall and septum) was snap-frozen in liquid nitrogen and stored at −80 °C.

2.3. Protein-extraction, Western blot analysis and antibodies

Heart tissue samples were lysed in radioimmunoprecipitation assay buffer, after which Western blot analysis was performed as described before (Brundel et al., 1999, 2001; Wiersma et al., 2017). Briefly, equal amounts of proteins in SDS-PAGE sample buffer were homogenized, by use of a 26G needle and syringe, before separation on 4–20% Precise™ Protein gels (Thermo Scientific, USA). After transfer to nitrocellulose membranes (Stratagene, The Netherlands), membranes were incubated with primary antibodies and subsequently with horseradish-peroxidase-conjugated secondary antibodies. Signals were detected by the Western Lightning Ultra (PerkinElmer, USA) method and quantified by densitometry with the software GeneGnome and GeneTools (SynGene, UK). Primary antibodies used were: rabbit polyclonal anti-HSF1 (#4356), rabbit polyclonal anti-LC3B (#2775), rabbit polyclonal anti-phospho-mTOR (Ser2448, #2971), rabbit polyclonal anti-phospho-eIF2α (Ser51, #9721, all Cell Signaling Technology, The Netherlands), rabbit polyclonal anti-HSP25 (ADI-SPA-801, Enzo Life Sciences, USA), rabbit polyclonal anti-GRP78/BiP (ab21685, Abcam, UK) and mouse monoclonal anti-β-actin (#sc47778, Santa Cruz Biotechnology, USA). Secondary antibodies used were horseradish-peroxidase-conjugated anti-mouse or anti-rabbit (Dako, Denmark).

2.4. Quantitative real time-PCR analysis

Total RNA from heart tissue samples was extracted using Trizol (Invitrogen, The Netherlands), according to manufacturer's instructions. First strand cDNA was generated by M-MLV reverse transcriptase and random hexamer primers (Promega, The Netherlands). Subsequently, cDNA was used as a template for quantitative real-time