Comparative Biochemistry and Physiology, Part C 140 (2005) 165–174

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Seasonal modulation of free radical metabolism in estivating land snails *Helix aspersa*

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Received 16 July 2004; received in revised form 18 January 2005; accepted 20 January 2005

Abstract

We investigated the regulation of free radical metabolism in *Helix aspersa* snails during a cycle of 20-day estivation and 24-h arousal in summer in comparison with estivation/arousal in winter-snails. In winter-snails (J. Exp. Biol. 206, 675–685, 2003), we had already observed an increase in the selenium-dependent glutathione-peroxidase (Se-GPX) activity in foot muscle and hepatopancreas and in the contents of hepatopancreas GSH-equivalents (GSH-eq=GSH+2 GSSG) during estivation compared with 24-h aroused snails. Summer-estivation prompted a 3.6-fold increase in Se-GPX activity in hepatopancreas, though not in foot muscle. Total-superoxide dismutase and catalase activities in hepatopancreas decreased (by 30–40%) during summer-estivation; however, no changes occurred in the activities of glutathione reductase, glutathione *S*-transferase and glucose-6-phosphate dehydrogenase in the two organs. GSH-eq levels were increased (by 54%) in foot muscle during estivation, but were unchanged in hepatopancreas. In contrast with winter-snails, oxidative stress markers (lipid peroxidation, carbonyl protein, and the GSSG/GSH-eq ratio) were unaltered during estivation/arousal in summer. These results demonstrate that seasonality modulates not only the absolute activities/levels of antioxidants (enzymes and GSH-eq) in *H. aspersa*, but also the regulatory process that controls the snail's antioxidant capacity during estivation/arousal. These results suggest that *H. aspersa* has an "internal clock" controlling the regulation of free radical metabolism in the different seasons.

Keywords: Estivation; Seasonality; Antioxidants; Glutathione; Oxidative stress; Lipid peroxidation

1. Introduction

Estivation of snails caused by seasonal lack of water, humidity or food is accompanied by a reduction in metabolic rate. The reductions in metabolic rate during estivation (up to 80–90% metabolic arrest depending on the species; Guppy and Withers, 1999) may translate into considerable energy savings in dormant animals compared with active ones. The biochemical changes leading to metabolic depression in land snails could include general alteration in phosphorylation pattern of proteins and regulatory enzymes, reduction in the overall rate of mitochondrial respiration, fuel oxidation (mostly carbohydrate and lipids) and protein synthesis, as well as alterations in levels of several protein-factors involved in the control of transcription and translation (Bishop et al., 2002; Pakay et al., 2002; Storey, 2002; Storey and Storey, 2004).

The levels of certain endogenous antioxidants [glutathione (GSH) and/or antioxidant enzymes] are also increased during estivation in the land snails *Otala lactea* and *Helix aspersa*, and in freshwater snails *Biomphalaria tenagophila* (Hermes-Lima and Storey, 1995; Ferreira et al., 2003; Ramos-Vasconcelos and Hermes-Lima, 2003). This

Abbreviations: CHE, cumene hydroperoxide equivalents; FOX, ferrous-xylenol orange; GR, glutathione reductase; GSH, glutathione reduced form; GSH-eq, glutathione equivalents (GSH-eq=GSH+2 GSSG); GSSG, glutathione disulfide; GST, glutathione *S*-transferase; G6PDH, glucose-6-phosphate dehydrogenase; ROS, reactive oxygen species; Se-GPX, selenium-dependent glutathione peroxidase; TBARS, thiobarbituric acid reactive substances; total-SOD, CuZn-plus Mn-superoxide dismutase.

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doi:10.1016/j.cca.2005.01.015

increased antioxidant capacity is believed to be an adaptive strategy to minimize the effects of reactive oxygen species (ROS) production during the resumption of normal metabolic rates (Hermes-Lima et al., 1998, 2001, 2004; Hermes-Lima and Zenteno-Savín, 2002). Arousal from estivation in land snails is generally accompanied by a transitory increase in oxygen consumption (Hermes-Lima and Zenteno-Savín, 2002; Storey, 2002), which may augment the mitochondrial production of ROS. Indeed, a transient increase in lipid peroxidation was observed in the hepatopancreas of *O. lactea* and *H. aspersa* within minutes following arousal (Hermes-Lima et al., 1998; Ramos-Vasconcelos and Hermes-Lima, 2003).

Oxidative stress has also been associated with recovery from metabolic depression in vertebrates. Bagnyukova et al. (2003) observed increased levels of oxidative damage to proteins, in organs of frogs *Rana ridibunda* during forced arousal from hibernation. Lushchak et al. (2001) showed increased hepatic lipid peroxidation in goldfish (*Carassius auratus*) after 1-h reoxygenation following anoxia exposure. Moreover, increased production of ROS was proposed to take place in hibernating Arctic ground squirrels (*Spermophilus parryii*) during awakening (and rewarming from 3 to 37 °C; see Hermes-Lima and Zenteno-Savín, 2002). In contrast, estivation in desert frogs was accompanied by a decrease in antioxidant capacity and augmented lipid peroxidation in several tissues (see Hermes-Lima et al., 2001).

Although free radical metabolism is clearly affected by metabolic depression, as far as we know, there is no evidence that the regulation of antioxidant capacity (which controls oxidative damage to tissues) during hypometabolism/arousal is seasonally modulated. Seasonal changes in free radical metabolism have been reported in selected tissues of rats (Sólar et al., 1995; Belló-Klein et al., 2000) and of three species of ground squirrels (Buzadzic et al., 1990, 1998; Blagojevic et al., 1998; Carey et al., 2003; Hermes-Lima et al., 2004). The seasonal modifications in the antioxidant profile (and capacity) have been related to specific adaptations against oxidative stress during hibernation/arousal cycles in ground squirrels (Hermes-Lima et al., 2004). Seasonal alterations in free radical metabolism were also observed in a cichlid fish (Wilhelm Filho et al., 2001a), in an estuarine polychaete (Geracitano et al., 2004) and in various mussel species (Viarengo et al., 1991; Solé et al., 1995; Power and Sheehan, 1996; Wilhelm Filho et al., 2001b).

Taking in consideration that previous estivation/arousal experiments with land snails *H. aspersa* were conducted in the Brazilian winter season (Ramos-Vasconcelos and Hermes-Lima, 2003), we decided to investigate the regulation of free radical metabolism in summer during a cycle of 20-day estivation followed by 24-h arousal. In a previous report, we discussed seasonal differences in glutathione peroxidase activity from selected organs of *H. aspersa* (Hermes-Lima et al., 2004). In this study, we made a full-length analysis of the activity of five antioxidant enzymes and glucose-6-phosphate dehydrogenase and levels of glutathione and markers of oxidative stress from estivating and arousing *H. aspersa* in summer. The results were then compared with those from winter-snails, revealing relevant seasonal differences in the modulation of free radical metabolism during estivation and arousal in *H. aspersa*.

2. Materials and methods

2.1. Chemicals

All the other reagents used were of analytical grade and are listed in a previous publication (Ramos-Vasconcelos and Hermes-Lima, 2003). All solutions were prepared with Milli-Q deionized water.

2.2. Animals

Brown garden snails *H. aspersa* were purchased from Heliário Araras (State of Rio de Janeiro, Brazil). The animals weighed 15–18 g and were kept in the laboratory (indoors; at 24–26 °C year-round, with occasional drops to 18–21 °C at night-time, especially in winter; see below) in glass containers with a 12:12 h light–dark cycle. The animals were fed lettuce sprinkled with ground chalk and sprayed with dechlorinated water at 20-day intervals, which also induces arousal in estivating snails (see below). For sampling purposes, the snails were killed by breaking their shells and the organs (foot muscle and hepatopancreas) quickly dissected out and frozen in liquid nitrogen. Organ samples were stored at −75 °C.

Drs. Armelle Ansart and Luc Madec (Université de Rennes 1, France) identified the snails used in this work (also used in previous studies: Ramos-Vasconcelos and Hermes-Lima, 2003; Hermes-Lima et al., 2004) as *H. aspersa maxima*.

2.3. Estivation/arousal experiments

Estivation was induced in the laboratory by removing water and food from the containers. Within 1 day, the animals retracted inside their shells and estivation was timed from that moment on. One group of snails was sampled after 20 days of continuous dormancy. Another group was sprayed with water, aroused and fed; this group was then sampled after 24 h.

These estivation experiments were conducted in our laboratory (indoor experiments, see above) in March 1997, January 1998 and March 1998, which corresponds to the rainy summer season in Brasília, located in mid-western Brazil [1000 m above sea level; average outdoor humidity and temperature for January–March in Brasília are 76% and 21–22 °C (INMET, 1961/1990)]. The results from