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Uric acid deposits and estivation in the invasive apple-snail, *Pomacea canaliculata*

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ABSTRACT

The physiological ability to estivate is relevant for the maintenance of population size in the invasive *Pomacea canaliculata*. However, tissue reoxygenation during arousal from estivation poses the problem of acute oxidative stress. Uric acid is a potent antioxidant in several systems and it is stored in specialized tissues of *P. canaliculata*. Changes in tissue concentration of thiobarbituric acid reactive substances (TBARS), uric acid and allantoin were measured during estivation and arousal in *P. canaliculata*. Both TBARS and uric acid increased two-fold during 45 days estivation, probably as a consequence of concomitant oxyradical production during uric acid synthesis by xanthine oxidase. However, after arousal was induced, uric acid and TBARS dropped to or near baseline levels within 20 min and remained low up to 24 h after arousal induction, while the urate oxidation product allantoin continuously rose to a maximum at 24 h after induction, indicating the participation of uric acid as an antioxidant during reoxygenation. Neither uric acid nor allantoin was detected in the excreta during this 24 h period. Urate oxidase activity was also found in organs of active snails, but activity shut down during estivation and only a partial and sustained recovery was observed in the midgut gland.

1. Introduction

Estivation is a state of behavioral quiescence and metabolic arrest which occurs in response to drought and/or high ambient temperature in several vertebrate and invertebrate taxa and may be associated with a profound metabolic depression (Guppy and Withers, 1999). The physiological mechanisms of estivation have been mostly studied in anurans (Amphibia) and land-living pulmonates (Gastropoda) (Hermes-Lima and Zenteno-Savin, 2002; Storey, 2002), where a critical aspect is the defense against the excess production of free oxygen radicals at the time of arousal from estivation. Hermes-Lima et al. (1998) pointed to the overall similarities between tissue hypoxia/re-oxygenation occurring during the estivation/arousal sequence of events in land-living gastropods, and those occurring after ischemic injury in man (as in myocardial infarction and stroke), in which reperfusion with oxygenated blood does not simply reverse the stress but instead triggers a series of post-ischemic oxidative injuries caused by oxyradicals (McCord, 2000; Young and Woodside, 2001; Rahman, 2007; Halliwell, 2009).

Several authors (Hermes-Lima et al., 1998; Hermes-Lima and Zenteno-Savin, 2002; Ramos-Vasconcelos et al., 2005; Nowakowska et al., 2009) have shown that different enzymatic mechanisms and glutathione, protect pulmonate gastropods from the damaging effects of re-oxygenation during post-estivation arousal. In contrast, uric acid has been shown to act as a non-enzymatic antioxidant (Ames et al., 1981; Becker, 1993) and it has been suggested that it may play such role in post-estivation arousal (Hermes-Lima and Storey, 1995b; Vega et al., 2007; Giraud-Billoud et al., 2008).

The apple-snail *Pomacea canaliculata* (Lamarck, 1822) (Architaenioglossa, Ampullariidae) shows an elaborate array of urate tissues distributed in lung, gill, coiled gut, midgut gland, testis, ampullary heart cavity and anterior kidney containing intracellular urate crystalloids (Vega et al., 2007; Giraud-Billoud et al., 2008). The latter study showed a sequential process of crystalloid formation and lysis, which occurs asynchronously in cells within the same tissue, suggesting an active turnover of uric acid in these cells.

Pomacea canaliculata is able to estivate in the field (d'Orbigny, 1847; Cowie, 2002), and the current study has dealt with changes occurring during experimental estivation and during post-estivation arousal in both uric acid and allantoin concentration in soft tissues of this snail, to test the hypothesis that uric acid and allantoin are involved in antioxidant mechanisms of the arousing snails. Allantoin is an oxidation product of uric acid that may be produced either spontaneously (when uric acid acts as an electron acceptor) or by urate oxidase (EC 1.7.3.3) catalysis.

2. Materials and methods

2.1. Animals and culturing conditions

Adult males and females (30–40 mm shell length) obtained from a cultured strain of *P. canaliculata* were used. All groups in this study

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were composed by an equal number of males and females. The original stock was collected at the Rosedal Lake (Palermo, Buenos Aires, Argentina) and voucher (ethanol preserved) specimens of the original population, were deposited at the collection of Museo Argentino de Ciencias Naturales (Buenos Aires, Argentina; lots MACN-In 35707 and MACN-In 36046, respectively). Temperature was regulated at 24–26 °C and artificial lighting was provided 14 h per day. Room relative humidity varied around 80%. Aquarium water was changed thrice weekly. Animals were fed ad libitum with a mixed diet made mostly of fresh lettuce, supplemented weekly with carp food pellets, desiccated and powdered eggs and toilet paper.

2.2. Mortality rate and body mass changes during experimental estivation and after arousal

The animals were induced to estivate by leaving them on a dry surface in the culture room. Day 0 of estivation of each snail was defined as the day when the operculum appeared firmly attached to the shell aperture (see Results section). At that time each snail was put in an individual dry container in the culture room.

In one set of observations, the mortality rate was recorded on days 2, 15, 30, 45, 60 and 75 of experimental estivation (independent groups of N = 24 for each day; this was made since in previous observations the snails appeared extremely sensitive to handling during estivation). Four of the dead snails showed fly puparia in their proximity, and these were collected and kept in close containers (to prevent the adults to escape after emergence) and they were later fixed in 70% ethanol for taxonomic identification (see Acknowledgements). In a second set, body mass changes during experimental estivation were recorded for 18 animals, by weighing them on day 0 (as defined above) and on days 2, 15, 30 and 45 thereafter. Body mass losses were expressed as percent of the initial value on Fig. 1B. In a third set of observations, arousal was induced in 30 animals after 45 days of estivation by transferring each snail to an individual vessel with 45 mL of tap water, so that the animals were only partly submersed. Time 0 of arousal was defined as the time of operculum detachment, and the animals were drained and weighed 20 min, and 24, 48, 72 and 96 h thereafter (N = 6 per group). A large piece of fresh lettuce was offered 90 min after water exposure. Body mass recovery was computed for each snail as the body mass difference between day 0 of estivation and the corresponding post-arousal value. These results were expressed as percent recovery on Fig. 1C.

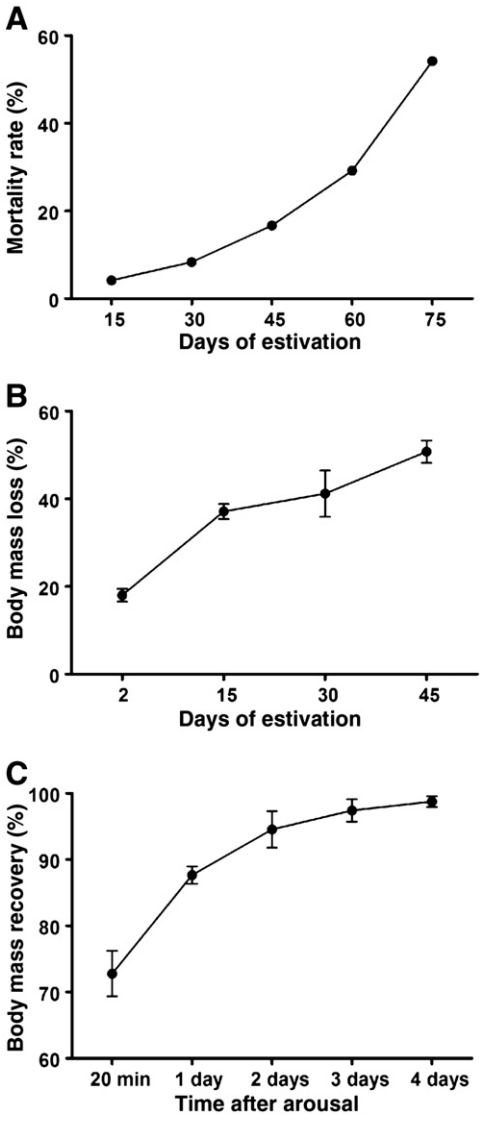

Fig. 1. Mortality rate and body mass changes during experimental estivation and after arousal in *P. canaliculata*. A. Mortality rate during experimental estivation (N = 72). B. Body mass loss during experimental estivation (mean % change ± SE, N = 18). C. Body mass recovery after induction of arousal (mean % change ± SE, N = 30).

2.3. Behavioral observations

Eighteen animals which were induced to estivate by leaving them on a dry surface were observed at 2-h intervals during the first 10 h, and at 24, and 48 h after induction. Also, 18 animals that were estivating for 45 days, were induced to arouse by transferring them to water containing vessels. A large piece of fresh lettuce was added to each vessel 24 h after induction. Also, behavioral observations of the arousing snails were made at 5, 10, 20, 30 and 50 min, and at 5 and 10 h after induction.

2.4. Experimental groups and sacrifices

Four groups (six animals each) were set for exploring the changes in uric acid and allantoin concentration in the soft tissues of control, estivating and aroused animals. The control group was kept in the standard culturing conditions, while the other three groups were induced to estivate. One of the latter groups was sacrificed in the morning of day 45 of estivation, while the other two groups were aroused from estivation on that morning, and were sacrificed either 20 min or 24 h after the operculum was detached from the shell (see Results section).

Also, 24 h-excreta were collected from six active control snails and from six estivating snails that were exposed to water to induce arousal. For such purpose, they were weighed (the active snails were previously drained) and each one was placed in a vessel containing 45 mL of tap water with no food, and with the addition of penicillin-G 0.6 g/L and streptomycin sulfate 0.6 g/L, to prevent possible bacterial action on the excreted compounds, as described in Vega et al. (2007). Arousal of the estivating snails was induced by water exposure in these vessels, and 24 h later the animals were removed and water with the excreta was thoroughly mixed and then centrifuged at 3000 g for 20 min at 4 °C. Aliquots (2.5 mL) of the obtained supernatants (soluble excreta) were collected and frozen while the precipitates (particulate excreta) were homogenized for 10 min at 4 °C in 1.5 mL of 0.5% lithium carbonate (Ultra-Turrax® homogenizer; IKA® Werke GmbH & Co., Germany), and centrifuged at 3000 g for 20 min at 4 °C. The supernatants were aliquoted (2.5 mL) and kept frozen until uric acid and allantoin determinations were made.