Brown garden snails (Helix pomatia L.), spend cold winter seasons as well as dry and hot summer seasons in torpor, which is characterized by a strong reduction of metabolic rate (Guppy & Withers, 1999). At the end of winter torpor the snails enhance the capacity of their antioxidant defence system to prevent oxidative stress, which accompanies return from hypometabolism to activity (Nowakowska et al., 2009a). The augmented antioxidant defence, including synthesis of glutathione and glutathione-related enzymes, has also been shown in Cornu aspersum (Ramos-Vasconcelos & Hermes-Lima, 2003; Ramos-Vasconcelos, Cardoso & Hermes-Lima, 2005) during dormancy/activity cycles. On the other hand, active H. pomatia exhibit a high level of antioxidant defence during summer (Nowakowska et al., 2009b), which can be regarded as a preadaptation to unpredictable, irregular episodes of aestivation. Because the capacity of antioxidant defence in active snails during spring was lower than that recorded at the same time in their counterparts continuing in torpor (Nowakowska et al., 2009a), we wondered how they would respond to aestivation, induced experimentally, in this period. It must be stressed that early spring weather in the north temperate zone of central Poland can occasionally be hot and dry enough to induce aestivation. Therefore, the ability of snails to survive under such stressful conditions should be determined by the adjustment to sudden environmental challenges. We examined the ability of H. pomatia to modulate their antioxidant defence in response to dehydration-induced torpor during early spring and we compared their response with that previously recorded in torpid snails during summer (Nowakowska, Caputa & Rogalska, 2011).

The capacity of antioxidant defence was estimated as activities of antioxidant enzymes (catalase, CAT; total glutathione peroxidase, GPX; selenium-dependent glutathione peroxidase, Se-GPX and glutathione transferase, GST), as concentration of reduced glutathione (GSH) and as end products of lipid (thiobarbituric acid reactive substances, TBARS) and protein (carbonyl) peroxidation during the aestivation/arousal cycle in the kidney, hepatopancreas and foot of 30 adult specimens of H. pomatia. The snails were collected from their natural habitat in central Poland (53°02’N, 18°35’E) as soon as they emerged from winter torpor during spring (24 April) and they were immediately deprived of food and water to induce aestivation (n = 18). The aestivating snails were used in one of three experimental treatments: (1) after 3 weeks of aestivation (n = 6), (2) just after arousal from aestivation (n = 6) and (3) 24 h postarousal (n = 6). Snails in groups 2 and 3 were sprayed with water to interrupt aestivation, which happened within a few minutes. The control group (n = 6) consisted of active snails, examined just after being collected from the field. In addition, snails in additional control group (n = 6) collected in the field together with the experimental snails, were put into a cage, sprayed with water every day and fed ad libitum. These were used to examine the effect of laboratory conditions on the antioxidant defence system. The methods used to examine activities of antioxidant enzymes and concentrations of GSH, TBARS and CP have been described in detail elsewhere (Nowakowska et al., 2009a, b; Nowakowska, Caputa & Rogalska, 2010, 2011; Nowakowska et al., 2014). The results were analysed by two-way ANOVA (experimental conditions x organs as factors), followed by Tukey-Kramer post hoc test.

The investigation showed that snails forced to aestivate in early spring did not show signs of preparatory changes for arousal-induced oxidative stress, such as increased level of CAT, which was observed in summer-torpid snails (Nowakowska et al., 2009b; Nowakowska et al., 2010, 2011), but forced aestivation led to a strong decrease in the kidney’s enzyme activity (Fig. 1). In both spring (Fig. 1) and summer aestivation (Nowakowska et al., 2011) the lowest activities of CAT were recorded in the foot and were significantly different (P < 0.001) from those in the hepatopancreas and kidney, but in spring there was no compensatory increase in activity of total GPX in the foot, which has been recorded in summer throughout both torpor and activity cycles and under control conditions (Nowakowska et al., 2011). In the present paper, activity of total GPX was found to be organ-dependent, but it was unaffected by experimental conditions; during the spring, the aestivation/arousal cycle activity of the enzyme remained relatively stable (except for transient changes in postarousal activity of the enzyme in the kidney indicated by the post hoc test, see Fig. 1). Our previous studies showed some discrepancies in activity of total GPX in summer-aestivating snails; in one case (Nowakowska et al., 2009b) there was a transient, highly significant increase in the activity in the kidney of snails freshly aroused from aestivation, but in two other cases (Nowakowska et al., 2010, 2011) aestivation/arousal cycles did not influence activity of the enzyme in the kidney or that in two other organs. In the present study, activity of Se-GPX was influenced by experimental conditions and organ type, and the highest activity was recorded in the foot (Fig. 1). Moreover, in all experimental groups, activities of Se-GPX in
spring were higher than those recorded in summer (Nowakowska et al., 2011). On the other hand, activities of GST in spring (Fig. 1) were lower than those in summer (Nowakowska et al., 2011). Figure 1 shows that high activities of Se-GPX in the foot are associated with low activity of CAT in the organ. This supports idea that Se-GPX is the most important antioxidant enzyme in land snails (Ramos-Vasconcelos & Hermes-Lima, 2003). In both spring (Fig. 1) and summer (Nowakowska et al., 2011) the highest activities of GST were recorded in the hepatopancreas of snails aroused from aestivation. Figure 2 shows that concentrations of glutathione are unaffected by the torpor/activity cycle, as has also been recorded in summer (Nowakowska et al., 2011). However, there was a transient increase in 24-h postarousal concentration of the compound in the kidney, which was indicated in the present study by the post hoc test. The stability of the compound concentration is compatible with the following properties of GSH: (1) it acts as a hydrogen donor for Se-GPX and GST (Storey, 1996) and, therefore, its concentration should remain as constant as possible and (2) its accumulation is advantageous, since its production is less costly than synthesis of antioxidant enzymes (Meister & Anderson, 1983). The relative stability of the

**Figure 1.** Activities of catalase and glutathione-related enzymes in three organs of *Helix pomatia*: forced to aestivate for 3 weeks (E), just aroused (A), after being aroused for 24 h (24H) and in two control groups consisting of snails active in the laboratory (LAB) and outdoor-active snails (OUT). Values are presented as mean ± SE. Abbreviations: a, significantly different from the values recorded in the same organs in LAB group; b, significantly different from the same organ in OUT group; c, significantly different from the values in the same organs in E group; d, significantly different from the values in the same organs in A group; *, significant differences between organs in same experimental groups (***P < 0.001; **P < 0.01; *P < 0.05).

**Figure 2.** Concentrations of glutathione in three organs of *Helix pomatia*. Abbreviations and conventions as in Figure 1.
antioxidant system was associated with insignificant changes of concentration of TBARS (Fig. 3, left panel). On the other hand, concentration of CP (Fig. 3, right panel) was strongly affected by experimental conditions. The largest, albeit rather modest, level of protein damage was recorded in the foot of active 24-h postarousal and laboratory control snails. Interestingly, concentrations of CP in aestivating snails were not different from those recorded in the outdoor control group. Altogether, H. pomatia is able to prevent oxidative damage to its lipids and proteins even under the extreme weather disturbances.

This study shows that H. pomatia modulates its defence mechanisms according to challenges of its environment. This suggests that activity of antioxidant enzymes varies depending on time after arousal from winter torpor. Accordingly, we conclude that snails are not only able to prepare their organs for oxidative stress imposed by a transition from winter torpor to the active state, but they can also cope with an excessive production of reactive oxygen species repeated a couple of weeks later.

ACKNOWLEDGEMENTS

This work was supported by grant no. N304 393238 from the Polish Ministry of Science and Higher Education.

REFERENCES


