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Hsp70 level in progeny of aging grasshoppers from variously polluted habitats and additionally exposed to zinc during diapause

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ABSTRACT

The hsp70 level in the bodies of 1st instars of grasshoppers *Chorthippus brunneus* from unpolluted (Pilica) and polluted (Olkusz, Szopienice) sites and additionally exposed to various doses of zinc during diapause and embryonic development prior to hatching were measured by Western blotting. The main aim of our work was to assess the relationship between the age of female grasshoppers originating from variously polluted habitat and the hsp70 level in their progeny. Possible reasons for population variation in hsp70 levels were discussed. The hsp70 level in the offspring's body depended on the place of origin. The strongest expression of hsp70 was found in the bodies of larvae hatching from the eggs laid by young females from Pilica (reference site). In contrast, a low initial level of hsp70 in larvae from polluted sites, especially in young females' progeny, was observed. The application of zinc during diapause influenced the hsp70 level in grasshopper larvae; however, the direction of the changes depended on the insects' place of origin. In larvae from the reference site, and also (but to a lesser degree) from Olkusz, the increase in the hsp70 level after zinc treatment was most pronounced. Whereas in grasshoppers from Szopienice, zinc (in 100 $\mu g\ g^{-1}$ dry weight of sand) did not change the hsp70 level, or (in 500 $\mu g\ g^{-1}$ dry weight of sand) caused a reduction in hsp70. The differences may result from maternal effects; however, possible adaptation also cannot be excluded. To confirm this statement further studies are needed.

1. Introduction

Heat shock proteins (hsps), also known as stress proteins (sps) or chaperons, are molecules the expression of which increases under stressful conditions. The stress can be caused by either endogenous or environmental exogenous factors. It has been known since the 1960 that the enhancement of the synthesis of the proteins is related to heat shock. The molecules may play an important role in the selection of individuals resistant to high temperature (Feder, 1997; Krebs and Feder, 1998; Feder et al., 2000). Further studies have shown that an increased synthesis of proteins may also be induced by UV radiation, free radicals, changes in pH, viral infections, metals and many other stressors. An increase in the level of damage to important molecules, including enzymatic proteins, and disorders in the functions of the organism are the final effect of exposure to the factors mentioned above. In such situations the beneficial action of heat shock proteins lays in, among others, prevention against denaturation and aggregation of proteins and their improper folding as well as facilitating the repair of destroyed proteins (Eckwert and Köhler, 1997; Köhler et al., 1999; Knigge and Köhler, 2000; Karouna-Renier and Zehr, 2003; Korsloot et al., 2004). Stress proteins are a large group of proteins that have been classified into families according to their molecular weight. Proteins from different families play various roles but, in general, they co-operate and complement one another. Hsp70 is the largest and relatively well known family of stress proteins. They are regarded as the most important defensive mechanism in the organism (Korsloot et al., 2004). It has been deeply analyzed whether shock protein expression could be useful as a biomarker of stress in tissues of animals exposed to heavy metals. While a positive correlation was found between hsp70 expression and metal concentration in food, soil or water, in experiments on animals reared under laboratory conditions, such an interpretation is much more complicated when considering animals living in polluted areas, where they are exposed to environmental multistress. It has been found that the hsp70 expression level might depend on the kind of metal, its concentration and length of exposure. The developmental stage of the animal, its age, nutritional state and, finally, possible acquired tolerance to the metal are equally important (Köhler et al., 1996; Eckwert and Köhler, 1997; Pyza et al., 1997; Köhler et al., 1999; Knigge and Köhler, 2000; Köhler et al., 2000; Karouna-Renier and Zehr, 2003; Arts et al., 2004).

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To inhabit an ecological niche burdened with stressors such as heavy metals, it is necessary to reach a kind of a compromise from an energy point of view. To protect an individual and prolong its life span, it seems beneficial to enhance the synthesis of shock proteins in order to minimize the negative effects of these stressors. This, however, involves energy expenditures. Thus, when the enhanced expression exceeds an optimal, profitable level, further enhancement may have negative effects. The question of the role of stress proteins should be reconsidered when taking into account the problem of aging and reproduction of specimens living under especially strong environmental stress. It can be expected that the negative effects of stressing factors such as heavy metals, acting in the organism, intensify with the process of aging. In general we may state that aging is in part an effect of the increased impact of prooxidative factors accompanied by a gradual decrease in antioxidative mechanisms (Canesi and Viarengo, 1997; Ashok and Ali, 1999; Sohal, 2002; Sohal et al., 2002; Barja, 2004; Lithgow, 2006). Thus (in general) aging in a polluted environment should proceed more quickly than in unpolluted habitats. This rule, however, may not necessarily be true for organisms that, due to long-term contact with toxins, have developed a tolerance to the specific stressing factor (Augustyniak et al., 2008a). Can the changes be expressed in the offspring of organisms exposed to stressors? If so, what might be the direction of the changes?

The main aim of this work is to describe the relationship between the age of grasshopper females, together with their habitat (variously polluted), and the hsp70 expression level in their offspring. Experiments performed during this study enabled us to test the following working hypotheses:

(i) The hsp70 level in the tissues of 1-day-old grasshopper larvae is the same, irrespectively of the habitat and mother's age. All individuals of the species at the moment of hatching have the same basal level of stress proteins. It can be induced only after exposure to environmental stressing factors. This reaction could be based on physiological changes. The individuals from these populations defend themselves through an enhanced synthesis of stress proteins, which can be perceived as better preparation for contact with stressors present in their habitats.
(ii) The hsp70 concentration in the body of insects originating from areas strongly polluted by metal is lower than in grasshoppers from the reference site. Long-term (multigenerational) contact with metals might have led to the selection of individuals of a decreased level of stress proteins, which can be regarded as a symptom of tolerance. The defense against the toxic effects of heavy metals may be achieved by other mechanisms.

In the second part of our work we wanted to investigate the impact of an additional stressing factor. We chose zinc. This metal is one of the most important pollutants in our study sites (Augustyniak and Migula, 2000; Łaszczyca et al., 2004; Augustyniak et al., 2005, 2006, 2008a). It is especially crucial for those organisms, whose lives are entirely or partly connected with soil. This is the case with grasshoppers. Many grasshopper species deposit egg pods directly into the soil. There they undergo the diapause and embryonic periods prior to hatching. That is why the degree of soil pollution is especially important for the development of this insect species (Devkota and Schmidt, 1999; Augustyniak et al., 2008a, b). Metals usually do not pass the egg's chorion easily; however, zinc can be taken up actively since it is essential for the proper functioning of an organism. Zinc plays an important catalytic and regulatory role. It is necessary for the proper functioning of numerous enzymes, signal transduction, DNA replication and transcription, and the stabilization of three-dimensional structure of various molecules (Takeda, 2000; Maret, 2005). However, an excess of zinc is toxic for organisms (Kim et al., 1999; Augustyniak et al., 2006, 2008a). Therefore, we decided to test whether an application of zinc during the diapause and embryonic periods might cause some significant changes in the hsp70 concentration in the bodies of grasshopper larvae.

We measured the hsp70 concentration in the bodies of grasshopper larvae originating from areas variously polluted by metals, and additionally exposed to zinc during the diapause and post-diapausal embryonic periods. To avoid the possible influence of other variables (different and/or contaminated diet, heat shock etc.), the measurements were performed immediately after hatching.

2. Materials and methods

2.1. Insects

Chorthippus brunneus (Acrididae) is a common grasshopper species that inhabits sunny sites. The species easily settles at areas that have been changed by human activity. It is found in natural ecosystems (meadows, fields), as well as on farms and in pastures and waste heaps near metal smelters (Rabitsch, 1995; Baldi and Kisbenedek, 1997; Augustyniak and Migula, 1996, 2000; Devkota and Schmidt, 2000; Augustyniak et al., 2005, 2006, 2008a). *C. brunneus* is a very fertile species. The number of eggs laid by females depends on their age, climatic conditions, food availability, and the density of individuals in the population (Monk, 1985; Cherrill and Begon, 1989; Willott and Hassall, 1998; Augustyniak et al., 2008a). Diapause is obligatory; however, it can be broken under laboratory conditions (Moriarty, 1969).

2.2. Study area

The study sites are located in Upper Silesia (Southern Poland). Two polluted sites, Olkusz and Szopienice, located near metal smelters, were chosen. The reference site (Pilica) is far away from large industrial plants and smelters. Within a distance of 1 km from the meadow in Szopienice there is the mining-metallurgic smelter 'Szopienice'. The smelter, established in 1834, used to be an important producer of non-ferrous metals (mainly zinc and lead) and the biggest producer of cadmium in the world. 170 years of industrialization have resulted in heavy metal pollution of the soil, air and water. The level of acceptable accumulation of various metals in the soil near Szopienice has been greatly exceeded (Kucharski, 1993). Within 2.5 km of the meadow site in Olkusz there is the metal-metallurgic smelter 'Boleslaw', established much later, in 1956. Zinc and lead ores are excavated and smelted there to this day. Moreover, in the close vicinity of the two polluted sites, there are numerous smaller industrial plants, mines and smelters. The reference site (Pilica) is located in a meadow far from industrial areas (over 30 km from the 'Boleslaw' smelter) close to the town of Pilica. The Pollution Index (PI) for all the study sites is 22.7 (Szopienice), 12.1 (Olkusz) and 0.53 (Pilica). The PI was calculated using the following formula:

$$\mathrm{PI} = \frac{C_1}{C_{\mathrm{p}1}} + \frac{C_2}{C_{\mathrm{p}2}} + \cdots + \frac{C_n}{C_{\mathrm{p}n}}$$

where C_1, C_2, …, C_n are the concentrations of particular contaminants in a given area and $C_{\mathrm{p}1}$, $C_{\mathrm{p}2}$, …, $C_{\mathrm{p}n}$ are the maximal permissible levels of contaminants (Augustyniak and Migula, 2000). Data concerning the type of soil, its pollution, pH and the plant associations of each of the sites were published by Augustyniak and Migula (2000), Stone et al. (2001), Łaszczyca et al. (2004), Migula et al. (2004), Wilczek et al. (2004), Augustyniak et al. (2005) and Cabala and Teper (2007).