



Apoptotic and necrotic changes in the midgut glands of the wolf spider *Xerolycosa nemoralis* (Lycosidae) in response to starvation and dimethoate exposure



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ARTICLE INFO

Article history:

Received 19 June 2013

Received in revised form

16 September 2013

Accepted 25 September 2013

Available online 15 January 2014

Keywords:

Apoptosis
Dimethoate
Necrosis
Spiders
Starvation

ABSTRACT

In the present study, the intensity of degenerative changes (apoptosis, necrosis) in the cells of the midgut glands of male and female wolf spiders, *Xerolycosa nemoralis* (Lycosidae), exposed to natural (starvation) and anthropogenic (the organophosphorous pesticide dimethoate) stressors under laboratory conditions were compared. The spiders were collected from two differentially polluted sites, both located in southern Poland: Katowice–Welnów, which is heavily polluted with metals, and Pilica, the reference site. Starvation and dimethoate treatment resulted in enhancement of apoptotic and necrotic changes in the midgut glands of the spiders. The frequency of degenerative changes in starving individuals was twice as high as in the specimens intoxicated with dimethoate. The percentage of apoptotic and necrotic cells was higher in starving males than in starving females. A high intensity of necrotic changes, together with increased Cas-3 like activity and a greater percentage of cells with depolarized mitochondria, were typical of starving males from the polluted site. The cell death indices observed in females depended more strongly on the type of stressor than on previous preexposure to pollutants.

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1. Introduction

Spiders are regarded as important controllers of numerous insect populations in meadow, forest and agricultural ecosystems. As polyphagous and predatory invertebrates, spiders are potentially exposed to chemical stressors, especially heavy metals, because they are included in the group of macroconcentrators of these xenobiotics (Dallinger, 1993). Ecological assessments of arachnocenoses in degraded areas show that the quantities of spiders remain unchanged, but their species compositions are usually altered. This situation may result from species-specific differences in spider sensitivity to specific environmental stressors and, consequently, ease competition in this arthropod group (Migula et al., 2013).

Although the concept of stress can be considered at various levels of biological organization, the most common understanding of this issue focuses on individual organisms. In publications addressing this question, the behavioral and physiological reactions of spiders to

various stressing factors, e.g., metals (Deeleman-Reinhold, 1990; Hendrickx et al., 2003a), pesticides (Hof et al., 1995; Amalin et al., 2000; Nyffeler and Sunderland, 2003), temperature (Pulz, 1987; Schmalhofer, 1999) or starvation (Anderson, 1974; Nentwig, 1987; Li and Jackson, 1996; Pedersen et al., 2002), have been described. However, current knowledge of midgut gland structure and ultrastructure (Klann and Alberti, 2010; Lipovsek et al., 2002, 2004) as well as the cellular reactions of spiders in response to stress, especially in relation to laboratory exposure of spiders to factors that create environmental pressure, is limited.

The effects of stressing factors appear first at the molecular level and involve changes in polypeptide synthesis, the oxidation and denaturation of protein structures, DNA damage, intracellular respiration disorders and energy generation processes, among others. Consequently, cellular structures disintegrate, and metabolic processes are inhibited. In specific situations when the extent of damage is great, the cell dies following apoptotic and/or necrotic reactions. Under physiological conditions, apoptotic mechanisms are responsible for achieving the proper balance between the proliferation intensity and the rate of elimination of destroyed or unnecessary cells (Zakeri and Lockshin, 2002). Disturbances in this process caused

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by, e.g., environmental stressors may cause a decline in the physiological condition of an organ and, ultimately, of an organism. Apoptosis can be stimulated by various factors, such as temperature (Gorman et al., 1999), chemical substances (Mizutani et al., 2002; Tada-Oikawa et al., 2003; Gregorc and Ellis, 2011) or a deficiency of metabolic substrates (Kiesslich et al., 2005). Due to such correlations, this type of cell death is regarded as a sensitive and early index of chronic stress (Piechotta et al., 1999; Sweet et al., 1999). Recent studies have indicated a strategic role of mitochondria in the initiation of cell death (Fernandez-Checa, 2003; Orrenius, 2004). For this reason, measurements of changes in the mitochondrial potential ($\Delta\psi_m$) are considered a good indicator of the energetic and physiological condition of cells, tissues and whole organs (Saleh et al., 2003). Thus, this parameter can be applied in ecotoxicological studies as an efficient biomarker of the early subcellular effects of the action of chemicals in cells.

Necrotic cell death, unlike apoptosis, is a passive process. Necrosis covers whole cell sets. It is initiated by various physical, chemical and biological factors that, acting at a proper intensity within a very short period of time, initiate a number of morphological changes, loss of osmotic pressure and swelling of cells (McCall, 2010). However, this type of cell death can also be activated as secondary necrosis (programmed cell death) (Proskuryakov et al., 2003; Rost-Roszkowska et al., 2008).

The factors increasing the risk of reactive oxygen forms generation can also be potential stimulators of cell death. Depending on their concentrations in cells, the process of cell death follows an apoptotic or necrotic pathway. Apoptosis mainly results from oxidative stress of a lower intensity, whereas necrosis is stimulated by high oxidative stress (Proskuryakov et al., 2003).

Knowledge about cell death mechanisms in spiders is especially scarce. In analyses of the genetic control of programmed cell death performed in a single spider species, *Araneus ventricosus*, the cloning and phylogenesis of cDNA encoding a DAD1 homolog (defender against apoptotic cell death 1) were described. This protein was found to inhibit apoptosis in response to exposure of the spiders to low and high temperatures (Lee et al., 2003). In another study, it was revealed that the percentages of apoptotic cells in the midgut glands of mature females of the actively hunting spider *Pardosa lugubris* (Lycosidae) and web-building spider *Agelena labyrinthica* (Agelenidae), measured via a flow cytometry technique, were nearly ten times lower than that detected via histological methods in the hepatopancreas of the snail *Helix pomatia* (1.1 percent) but over ten-fold higher than in mouse liver (0.01 percent) (Chabicovsky et al., 2003, 2004). In that study, differences were also found in the cellular responses to thermal shock and/or organophosphorous pesticides between the studied spider species. The wolf spider *P. lugubris* shows a low frequency of apoptotic or necrotic changes in the midgut glands, whereas under the same experimental conditions, *A. labyrinthica* shows an increased percentage of cells dying through apoptotic and/or necrotic processes (Wilczek, 2005).

Xerolycosa nemoralis (Lycosidae) is a spider species that is abundant in polluted areas (Majkus, 1988). Comparative studies conducted in spiders collected at heavy metal-polluted sites have shown that the levels of Cd, Cu, Zn and Pb in their bodies are higher than in other spiders collected from the same environments (Wilczek et al., 2005, 2008). It has also been demonstrated

that, irrespective of the pollution levels at study sites, male *X. nemoralis* accumulate greater amounts of heavy metals in their midgut glands than females of this species. Additionally, the percentage of cells dying through necrotic processes in the midgut glands of male spiders is higher than in the midgut glands of females. In contrast, apoptotic pathways prevail in females (Wilczek et al., 2008). Analysis of the antioxidative responses of male and female *X. nemoralis* exposed to prooxidative factors of either an anthropogenic (dimethoate) or natural character (starvation and high temperature) under laboratory conditions indicated that the adaptive/compensative strategies that enable spiders to survive chronic stress differ between the sexes (Wilczek et al., 2013).

Considering the results of our previous studies on *X. nemoralis*, in the present study, comparisons were made between the intensity of apoptotic and necrotic changes as well as the quantitative changes in cells with depolarized mitochondria and the levels of caspase-like protease (Cas-3) in the cells of the midgut glands in response to starvation and treatment with the organophosphate pesticide dimethoate. Due to the strategic role of the midgut glands in digestion and detoxification processes, the analysis of selected cellular parameters was limited to this organ. The aim of the present study was also to determine whether, in relation to the analyzed parameters, female and male spiders that are chronically intoxicated with heavy metals in their habitats differ in their reaction to additional stressors compared to individuals from a reference site. The results may be helpful in the assessment of the analyzed parameters as biomarkers of the exposure of spiders, as small terrestrial predators, to such stressors.

2. Materials and methods

2.1. Study sites

Adult females and males of *X. nemoralis* were hand collected from two differentially polluted areas in southern Poland: Pilica, the reference site, and Katowice-Welnowiec, a post-metallurgic waste heap. The site treated as a reference is located in the Pilica commune (50°28' N, 19°39' E, silesian voivodship), at a distance of 30 km from any large industrial plants. Welnowiec (49°04' N, 18°55' E), the zinc-lead heap, is situated within the town of Katowice in Upper Silesia. The heap is composed of waste material originating from complex zinc and lead ore enrichment processes, and it consists mainly of dolomites, clays and toxic silts (Franiel and Więski, 2005). The concentrations of Zn, Cd, Cu and Pb in the soils of the study sites are presented in Table 1.

2.2. Spider species

The analyses conducted on adult females and males of the active hunting spider *X. nemoralis* (Lycosidae). *X. nemoralis* is a palearctic species that is abundant in northern Europe. Individuals of this species inhabit sunny sites with low vegetation as well as heathlands, grasslands, woodlands and clearings. Adults (♀4.5–7.5 mm, ♂4.5–6.0 mm) appear towards the end of May and in June. The spiders of this species hunt actively, sensing the vibrations of the ground caused by the movements of a potential victim (Varol et al., 2006; Wilczek et al., 2013).

Both male and female spiders were collected by hand into glass tubes. In the laboratory, individuals were kept in plastic containers in a rearing chamber under standard light (14 L:10 D), temperature (L: 25 °C, D: 15 °C) and humidity (70 ± 10 percent) conditions. The spiders in each experimental group received drinking water. Access to food (2nd and 3rd instars of the cricket *Acheta domestica* and adults of the fruit fly *Drosophila melanogaster*) depended on the experimental group.

Table 1

Mean concentrations of metals ($\mu\text{g g dry weight}^{-1}$) ± standard deviation (SD) in the humus layer from Welnowiec (polluted site) and Pilica (reference site).

Material	Sites	Cd	Pb	Cu	Zn
Humus	Welnowiec	6.4 (± 0.4)	445.3 (± 34.1)	64.8 (± 33.6)	1201.0 (± 177.1)
	Pilica	1.1 (± 0.4)	18.2 (± 1.6)	3.5 (± 0.9)	19.0 (± 1.8)

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