Abstract

The insect yolk precursor vitellogenin is a lipoglycoprotein synthesised and stored in the fat body and secreted into the hemolymph. In honey bees, vitellogenin displays crucial functions in hormone signalling, behavioural transition of nurse bees to foragers, stress resistance and longevity in workers. Plant protection products (PPPs) such as neonicotinoids, pyrethroids and organophosphate alter transcriptional expression of vitellogenin. To assess PPP-induced alterations on protein level, we developed a rabbit polyclonal vitellogenin antibody. After characterization, we assessed its specificity and vitellogenin levels in different tissues of worker bees. The vitellogenin antibody recognized full-length 180 kDa vitellogenin and the lighter fragment of 150 kDa in fat body, hemolymph and brain. In hemolymph, a band of approximately 75 kDa was detected. Subsequent mass spectrometry analysis (LC-MS/MS) confirmed the 180 and 150 kDa band as vitellogenin. Subsequently, we evaluated vitellogenin expression in brain, fat body and hemolymph upon 24 h exposure of bees to 3 ng/bee to the neonicotinoid clothianidin. Full length vitellogenin was up-regulated threefold in the fat body and the 150 kDa fragment in the brain of exposed honey bees, while no alteration occurred in the hemolymph. Up-regulation of the vitellogenin protein by the neonicotinoid clothianidin goes in line with the previously shown induction of its transcript. We conclude that vitellogenin might serve as a potential biomarker for neonicotinoid exposure in bees.

Key words: Honey bees; vitellogenin; neonicotinoids; clothianidin; antibody characterization

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INTRODUCTION

Honey bees are wild or semi-domesticated insects that live in colonies with complex division of labor (Winston, 1987). Bee colonies consist of one egg-laying queen, the female worker caste, which includes nurse bees and foragers, and the male drones. In contrast to queens and drones, the life-histories of workers can be very plastic during adulthood - individuals change from one social care task to another (Winston and Fergusson, 1985). In summer, workers typically progress from nurse bees with in-hive tasks to foragers. In autumn, when brood production decreases, bees develop into so-called winter bees (Maurizio 1950). Besides physiological specialization enabling bees to perform different tasks, the three major worker types (nurse, forager and winter bee) differ markedly in life-span. Foragers, one type of worker bee, typically die within two weeks and are the shortest-lived individuals, whereas bees continuing nursing can have life-spans longer than 50 days. The longest-lived workers, winter bees, survive from late summer to next spring. Only the queen lives longer, surviving 2 to 3 years, and in some cases up to 5 years (Dukas 2008, Münch et al. 2013).

The transition of nurse bees to foragers is hormonally regulated by juvenile hormone and vitellogenin. During the life course of workers, vitellogenin levels in the hemolymph and fat body drop, and these reduced concentrations influence several aspects of the life history of bees (Münch and Amdam, 2010). Levels of vitellogenin in hemolymph and fat body are highest in the long-living winter bees (up to 60-90 μg/μL hemolymph), and lowest in short-living foragers (0-5 μg/μL hemolymph) (Seehuus et al. 2006).

The phospholipoglycoprotein (Wheeler and Kawooya 1990) vitellogenin is a female-specific egg-yolk precursor (Spieth et al. 1991), synthesized by most oviparous animals including insects for transfer into oocytes, where it serves as nutrition for embryos. Besides the full length 180 kDa protein, vitellogenin was reported to occur as a 150 kDa fragment (Seehuus et al. 2007) and a 40 kDa fragment (Havukainen et al. 2010) in honey bees. Vitellogenin