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Comparative intestinal esterases amongst passerine species: Assessing vulnerability to toxic chemicals in a phylogenetically explicit context

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HIGHLIGHTS

- Passerine birds display interspecific differences in intestinal esterase and lipase.
- Phylogeny affected species-specific variations of esterase and lipase activities.
- Dietary nitrogen positively changed intestinal lipase activity.
- Field monitoring of organophosphorus exposure should consider bird phylogeny.

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ABSTRACT

Inhibition of blood esterase activities by organophosphate (OP) pesticides has been used as a sensitive biomarker in birds. Furthermore, compared to mammalian vertebrates, less is known about the role of these enzyme activities in the digestive tracts of non-mammalian vertebrates, as well as the environmental and biological stressors that contribute to their natural variation. To fill this gap, we examined butyrylcholinesterase (BChE) and carboxylesterases (CbE) in the digestive tracts of sixteen passerine species from central Chile. Whole intestine enzyme activities were positively and significantly correlated with body mass. After correcting for body mass and phylogenetic effect, we found only a marginal effect of dietary category on BChE activity, but a positive and significant association between the percentage of dietary nitrogen and the mass-corrected lipase activity. Our results suggest that observed differences may be due to the dietary composition in the case of lipases and BChE, and also we predict that all model species belonging to the same order will probably respond differently to pesticide exposure, in light of differences in the activity levels of esterase activities.

1. Introduction

Toxicity and metabolism of organophosphorus (OP) pesticides involve four esterase enzymes, i.e., acetylcholinesterases (EC 3.1.1.7), butyrylcholinesterases (EC 3.1.1.8), carboxylesterases (EC 3.1.1.1) and phosphotriesterases (EC 3.1.8.1). In the nervous tissue, inhibition of acetylcholinesterase (AChE) activity by OP compounds is the primary mechanism of toxic action of this class of agrochemicals (Fukuto, 1990), whereas the butyrylcholinesterase (BChE) and carboxylesterase (CbE) activities act as an efficient non-catalytic and stoichiometric mechanism of detoxification (Sogorb and Vilanova, 2002; Wheelock et al., 2008; Masson and Lockridge, 2010). The active site of these esterases is phosphorylated by the 'oxon' metabolite (oxygen analogue of phosphorothioate OPs) to yield a stable enzyme-inhibitor complex. Likewise, the oxon metabolites can be hydrolysed by the phosphotriesterases (Vilanova and Sogorb, 1999).

Over the last two decades, inhibition of blood cholinesterase and CbE activities by OPs has been used as a sensitive biomarker in both invertebrate (Guilhermino et al., 2000; Domingues et al., 2010; Malagnoux et al., 2014) and vertebrate species (Thompson and Walker, 1988; Sanchez et al., 1997; Soler et al., 1998; Nunes, 2011). The non-lethal nature of this approach together with the high sensitivity of blood esterases to OP inhibition have been the determining factors leading to the adoption of these biomarkers in birds (Thompson, 1991; Sogorb et al., 2007; Cheke et al.,

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2012). Many studies have identified the body size, age, sex and diet of birds as potential interfering sources in the interpretation of the levels of blood and liver CbE and BChE activities. For example, Bush et al. (1973) documented that birds with the greatest body mass had high levels of hepatic esterase activities compared to the smallest individuals. This was, however, questioned by Roy et al. (2005) and Sogorb et al. (2007), who found a negative correlation of plasma CbE activity with body mass in raptors. Likewise, diet is another potential factor of interspecies variation in blood esterase activity. It has been reported that omnivorous and herbivorous bird species have high blood CbE activity compared to insectivorous species (Bush et al., 1973; Roy et al., 2005; Thompson et al., 1988). In addition, recently Ríos et al. (2014) compared the CbE activity of the small intestine in two passerine species, finding that the higher activity in the omnivorous species would allow it to better deal with fats from different sources.

The main route of OP exposure in birds is believed to be through ingestion of contaminated food (Smith et al., 2007), although some recent studies have shown that acute exposure to organophosphorus pesticides by dermal or respiratory surfaces is also an important uptake route (Cheke et al., 2012). Therefore, the presence of pesticide-metabolizing esterases in the gastrointestinal epithelium would be the first biochemical barrier against pesticide uptake. However, as far as we know, no study has examined the role of these enzyme activities in the digestive tracts of non-mammalian vertebrates as well as the environmental and biological stressors contributing to their natural variation of activity. With the aim of describing and identifying the main biological factors contributing to inter- and intra-species differences in intestinal esterase activities and to assess putative toxicological impacts, we determined the levels of both BChE and CbE activity in the small intestines of sixteen passerine species inhabiting and foraging within the Chilean agroecosystem, covering a wide range of diet and size. Because lipase activity can interfere with the measurement of CbE activity when the latter is assayed with short-chain esters, this digestive enzyme was also included.

The main questions that we aimed to answer were: (1) Does the hydrolytic activity of small intestine esterases correlates with diet in passerines under a phylogenetically explicit context? (2) Is there any relationship between enzyme activity and body mass? (3) Does phylogeny impact the expression of these enzymes? It is our expectation that the present study will provide some answers to these questions, so more reliable assessments of pesticide exposure in field may be performed.

2. Materials and methods

2.1. Bird capture and tissue preparation

Birds were sampled in the following localities in Central Chile: Quebrada de la Plata (33°30′S, 70°54′W) and San Carlos de Apoquindo (33°23′S, 70°30′W), both of which are characterized by a Mediterranean climate. We captured a total of 114 adult individuals belonging to 16 species of songbirds using mist nets. To avoid the putative effect of dietary switches exhibited by some species in winter (Lopez-Calleja, 1995), captures were performed from November to January (austral summer). The captures were undertaken with the permission of the Chilean Agricultural Service (SAG).

Immediately after capture, the birds were transported to the laboratory, weighed, sacrificed by CO_2 exposure to be dissected and organs removed. The small intestine was rapidly excised, flushed with ice-cold saline solution (0.9% NaCl), measured (±0.1 cm) and weighed (±0.001 g) before being placed in storage at −50 °C. For biochemical assays, tissues were thawed and the whole small intestine was homogenized in 20 vol of 0.9% NaCl for 30 s at 24000 rpm using an Ultra Turrax T25 homogenizer (Janke and Kunkel, Breisgau, Germany). The homogenates were centrifuged at 5000 rpm for 10 min and 4 °C, and the supernatant was finally stored at −50 °C.

2.2. Esterase activities

Carboxylesterase activity was measured on an Asys HiTech UVM340 plate reader (Asys HiTech GmbH, Eugendorf, Austria) using the substrates alpha-naphthyl acetate (α-NA) and 4-nitrophenyl acetate (4-NPA) (Sigma-Aldrich, Madrid, Spain). The reason for using these two substrates was the existence of multiple CbE isoforms generally occurring in animal tissue with marked variability in substrate preference and sensitivity to OP inhibition (Wheelock et al., 2008). Hydrolysis of α-NA was measured as per the method described in Thompson (1999). Briefly, the enzymatic activity was run for 10 min at 40 °C in a reaction medium (200 μL, final volume) composed of 25 mM Tris–HCl (pH 7.6), 2 mM α-NA and the sample. The formation of α-naphthol was stopped by the addition of 75 μL of 2.5% SDS in 0.1% Fast Red ITR/2.5% Triton X-100, and the absorbance of the naphthol–Fast Red ITR complex was read at 530 nm after allowing the solution to stand for 30 min at 22–23 °C in the dark. The specific activity was calculated using a molar extinction coefficient of $33.2 \times 10^3\ M^{-1}\ cm^{-1}$ for the naphthol–Fast Red ITR complex. Hydrolysis of 4-NPA by CE (4-NPA-CE) was determined as described by Chanda et al. (1997). The reaction mixture (200 μL, final volume) contained 20 mM 4-NPA, 0.1 M Tris–HCl (pH 8.0) and the sample. The reaction was initiated by addition of 10 μL of 20 mM 4-NPA. The 4-nitrophenol liberated was read at 405 nm and quantified by a calibration curve (5–100 μM). Butyrylcholinesterase activity was determined according to Wheelock et al. (2005) as adapted from Ellman et al. (1961). The reaction medium (200 μL, final volume) was made up of 0.1 M Tris buffer (pH 8.0) containing 320 μM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and the sample. The reaction was initiated by addition of 60 mM butyrylthiocholine iodide (BTCI, Sigma-Aldrich, Madrid, Spain), and the product was monitored at 412 nm for 2 min. Specific activity was calculated using a molar absorption coefficient of $14.15 \times 10\text{-}3\ M^{-1}\ cm^{-1}$ (Eyer et al., 2003).

2.3. Lipase activity

Lipase activity was determined according to Gupta et al. (2002) using 4-NPP as the substrate. The reaction medium (250 μL, final volume) consisted of 50 mM Tris-HCl buffer (pH 8.0) containing 0.4% (w/v) Triton X-100 and the sample. The reaction was initiated with the addition of 20 μL of 16.5 mM 4-nitrophenyl palmitate (4-NPP) in dimethylsulfoxide, and the product (4-nitrophenol) was monitored at 412 nm for eight minutes. Specific activity was calculated using an external calibration curve made with 4-nitrophenol.

Protein concentration of each sample was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as the standard. All enzyme activities were standardized by protein content of sample (IU mg^{-1} protein) and by gram of wet tissue (IU g^{-1} wet tissue). We also calculated the summed activity for each enzyme by using the total weight of the small intestine, and data are presented as total hydrolytic activity (IU min^{-1}; 1 IU = 1 μmol of substrate hydrolyzed per min under the experimental conditions in this study). Although it is usual to measure the activities of membrane-bound enzymes in isolated preparations (Martinez del Rio and Stevens, 1989), we used homogenates of intestinal tissue, which yield consistent data of the activity of brush-border enzymes (Martinez del Rio et al., 1995; Brzek et al., 2013). In addition, the inefficient recovery of enzymes from brush-border isolated vesicles (Vasseur, 1989), may result in an