Ecotoxicology and Environmental Safety 135 (2017) 10–16

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effect of diet on carboxylesterase activity of tadpoles (*Rhinella arenarum*) exposed to chlorpyrifos

A.M. Attademo [a,b,*], J.C. Sanchez-Hernandez [c], R.C. Lajmanovich [a,b], P.M. Peltzer [a,b], C. Junges [a,b]

[a] CONICET-FBCB-UNL, Pje. El Pozo s/n, 3000 Santa Fe, Argentina
[b] Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (FBCB-UNL-CONICET), Paraje El Pozo s/n, 3000 Santa Fe, Argentina
[c] Laboratorio de Ecotoxicología, Facultad de Ciencias Ambientales y Bioquímica, Universidad Castilla-La Mancha, Toledo, Spain

ARTICLE INFO

Article history:
Received 31 May 2016
Received in revised form
11 September 2016
Accepted 13 September 2016

Keywords:
Organophosphorus
Carboxylesterase
Acetylcholinesterase
Pesticide detoxification
Amphibians
Outdoor microcosm

ABSTRACT

An outdoor microcosm was performed with tadpoles (*Rhinella arenarum*) exposed to 125 μg L^{-1} chlorpyrifos and fed two types of food, i.e., lettuce (*Lactuca sativa*) and a formulated commercial pellet. Acetylcholinesterase (AChE) and carboxylesterase (CbE) activities were measured in liver and intestine after 10 days of pesticide exposure. Non-exposed tadpoles fed lettuce had an intestinal AChE activity almost two-fold higher than that of pellet-fed tadpoles. No significant differences were observed, however, in liver AChE activity between diets. Likewise, intestinal CbE activity – measured using two substrates, i.e. 1-naphthyl acetate (1-NA) and 4-nitrophenyl valerate (4-NPV) – was higher in tadpoles fed lettuce than in those fed pellets. However, the diet-dependent response of liver CbE activity was opposite to that in the intestine. Chlorpyrifos caused a significant inhibition of both esterase activities, which was tissue- and diet-specific. The highest inhibition degree was found in the intestinal AChE and CbE activities of lettuce-fed tadpoles (42–78% of controls) compared with pellet-fed tadpoles (< 60%). Although chlorpyrifos significantly inhibited liver CbE activity of the group fed lettuce, this effect was not observed in the group fed pellets. In general, intestinal CbE activity was more sensitive to chlorpyrifos inhibition than AChE activity. This finding, together with the high levels of basal CbE activity found in the intestine, may be understood as a detoxification system able to reduce intestinal OP uptake. Moreover, the results of this study suggest that diet is a determinant factor in toxicity testing with tadpoles to assess OP toxicity, because it modulates levels of this potential detoxifying enzyme activity.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) and carboxylesterase (CbE, EC 3.1.1.1) are two serine hydrolases actively involved in the toxicity of organophosphorus (OP) pesticides (Thompson and Richardson, 2004). The former is the main molecular target in the mechanism of acute toxicity of OPs, particularly when the 'enzyme-pesticide' interaction takes place in the nervous system (Fukuto, 1990), whereas CbE activity detoxifies OP compounds by phosphorylation of serine residues in the active site of CbEs (Sogorb and Vilanova, 2002). Nevertheless, the affinity of OPs for the active centre of AChEs or CbEs increases several orders of magnitude with the oxon-analog metabolites of OPs (Chambers et al., 2010). Many studies including a wide range of organisms have evidenced that CbE activity displays a higher sensitivity to inhibition by OPs than AChE activity (reviewed in Wheelock et al., 2008), which has led some authors to postulate that CbE activity plays a protective role against OP toxicity, acting as a molecular sink.

Both AChE and CbE activities have been long used as biomarkers of OP exposure in wildlife and laboratory toxicity testing (Nunes, 2010; Wheelock et al., 2008). In this context, some studies have examined the impact of biological variables on baseline activity of cholinesterases (ChEs) and CbEs in non-exposed organisms. In general, body size and diet have a significant impact in the inter-specific variations of plasma ChE and CbE activities in birds (Goldstein et al., 1999; Sogorb et al., 2007; Narvaez et al., 2015). For example, Roy et al. (2005) examined intra- and interspecific variations of plasma ChE and CbE activities in 729 European raptors covering 20 species. They found that normal activity of plasma esterases in some of the studied species varied with sex, age and body size. Likewise, sex-related differences in blood ChE activity were reported for the Australian agamid *Pogona vitticeps* (Bain

* Correspondence to: Faculty of Biochemistry and Biological Sciences, National University of Littoral (FBCBUNL), Paraje el Pozo s/n, 3000 Santa Fe, Argentina.
E-mail address: mattademo@hotmail.com (A.M. Attademo).

http://dx.doi.org/10.1016/j.ecoenv.2016.09.012
0147-6513/© 2016 Elsevier Inc. All rights reserved.

et al., 2004), and the tegu lizard *Tupinambis merianae* (Bassó et al., 2012). In birds, a relationship was determined between diet preference and CbE activity levels; esterase activity was frequently found to be higher in omnivores than in carnivores (Thompson et al., 1991). Similarly, an inverse relationship was observed between body size and plasma esterase activities in birds (Roy et al., 2005) and amphibians (Lajmanovich et al., 2008). Dietary lipids are also important inducers of CbE activity in mammals (van Lith et al., 1992), passerine birds (Ríos et al., 2014), and some invertebrate species (Mommsen, 1978; Prento, 1987); accordingly, this esterase activity seems to be actively involved in lipid metabolism.

To our knowledge, there are no data on the response of CbE and AChE activities to OP exposure in animals fed different diets. Because CbE activity seems to have a physiological role in lipid metabolism (Ross and Edelmann, 2012), we hypothesized that lipid-rich diets would cause an increase of CbE activity, resulting in a more efficient detoxification capability against OP exposure. Therefore, the aims of this study were 1) compare CbE activity in the intestinal tissue and liver of tadpoles fed lettuce (*Lactuca sativa*) and commercial pellets, and 2) to examine whether the induction of CbE activity was a mechanism of detoxification in animals exposed to chlorpyrifos. For this purpose, AChE activity was included in this study as an additional molecular target of OP toxicity. We selected tadpoles as test organisms because they are sensitive indicators of ecosystem deterioration by environmental pollutants (Waddle, 2006), given their permeable skin, unshelled (not amniotic) eggs, and a complex life cycle that exposes them to changes in both aquatic and terrestrial environments (Blaustein et al., 1994). Accordingly, some ecotoxicological studies with amphibians have suggested the measurement of certain enzymes involved in the mechanism of contaminant toxicity as important biomarkers to detect potential adverse effects of pesticides (Ossana et al., 2013; Robles-Mendoza et al., 2011; Güngördü and Uçkun, 2015; Güngördü et al., 2016). We expect that our results will provide valuable information about the impact of diet on OP toxicity, which may be of concern in regulatory toxicology (toxicity testing and ecological risk assessment framework).

2. Materials and methods

2.1. Test species

Rhinella arenarum tadpoles were used as model test organisms. This anuran species has an extensive neotropical distribution (Cei, 1980; IUCN, 2010), occurring in forests, wetlands, agricultural lands, and urban territories (Attademo et al., 2005). In Argentina, this toad species is categorized as "not threatened" (Vaira et al., 2012) and is widely distributed in the provinces of Buenos Aires, Formosa, Chaco, Corrientes, Santiago del Estero, Entre Ríos, and Santa Fe. Eggs were collected from temporary ponds in natural floodplains of the Paraná River (31° 11′ 31″ S, 60° 9′ 29″ W, Argentina) with authorization of the Ministerio de Aguas, Servicios Públicos y Medio Ambiente (Santa Fe Province, Argentina). This site has not undergone pollution episodes nor has it been treated with chemical or biological pesticides (Attademo et al., 2014, 2015). The eggs were transported to the laboratory and maintained in dechlorinated tap water (pH = 7.4 ± 0.05; conductivity = 165 ± 12.5 μmhos cm^{-1}; dissolved oxygen = 6.5 ± 1.5 mg L^{-1} hardness = 50.6 mg L^{-1} $CaCO_3$ at 22 ± 2 °C). They were allowed to develop until tadpoles reached Gosner stage 26–27 (Gosner, 1960; approximately 5 days). A total of 32 tadpoles (total length = 16.67 ± 1.08 mm, weight = 0.054 ± 0.01 g) were used in this study.

2.2. Experimental design

An in vivo toxicity assay was performed in outdoor microcosms located at the Facultad de Bioquímica y Ciencias Biológicas (Universidad Nacional del Litoral, Santa Fe, Argentina), under an environmental temperature regime (24–26 °C), and a natural light/dark cycle (austral summer). Microcosms consisted of 20-L fishbowl enclosures covered with iron frames and fitted with 2-mm mesh to avoid predation or oviposition by insects or other anuran colonists. Treatment groups (n = 8 individuals per treatment) were: 1) tadpoles fed lettuce, 2) tadpoles fed pellets, 3) tadpoles fed lettuce and exposed to chlorpyrifos, and 4) tadpoles fed pellets and exposed to chlorpyrifos. Commercial pellets (Vita*Fish* brand, Santa Fe, Argentina) were composed of 44% proteins, 13.4% lipids, 15.2% minerals, 5.3% Ca, and 2.2% P. Lettuce was obtained from a local market; according to data from the USDA National Nutrient Database for Standard Reference (https://ndb.nal.usda.gov/ndb/foods) lettuce is composed of 1.36% proteins, 0.15% lipids, 3.0% minerals, 0.036% Ca, and 0.029% P. Edible portions of lettuce were boiled for a few minutes before use. Both food types were added to microcosms every two days. The amount of food was calculated following mass specific rations provided by Alford and Harris (1988). The OP pesticide used in this study was an emulsified formulation of chlorpyrifos named Tauro® LO (48% w/v chlorpyrifos) at a nominal concentration of 125 μg L^{-1}. This chlorpyrifos concentration was chosen because it is often included in the range of test concentrations in toxicity testing (Vera Candioti et al., 2014), although it was high compared with concentrations detected in streams receiving pesticides from soy-bean crops (Jergentz et al., 2005; Bonansea et al., 2013). Although we are aware that this pesticide concentration is unlikely to occur in surface waters receiving chlorpyrifos input by runoff, it might represent the worst-case scenario (e.g., flooding of agricultural areas or cumulative concentration after consecutive short-term pesticide applications). Tadpoles (5 d-old larvae, Gosner stage 33–35) were exposed to the pesticide and diet regimes for 10 days. Water was replaced with freshly prepared chlorpyrifos solution at the middle of the toxicity assay because of rapid degradation of chlorpyrifos in outdoor macrocosm (Mazanti et al., 2003).

Control and treated larvae were killed according to criteria of ASIH et al. (2011), and with the approval of the animal bioethics committee of the Faculty of Biochemistry and Biological Sciences (Acta 03/12). Each larva was immediately dissected along the mid-ventral line, and digestive organs (intestine and liver) were carefully removed, washed in distilled water and placed on filter paper to remove excess fluids. Tissue samples were stored at −80 °C until biochemical analyses.

2.3. Enzyme assays

Intestine and liver were homogenized (20% w/v) on ice-cold 25 mM Tris-HCl buffer (pH = 8.0) containing 0.1 Triton X-100 and using a polytron. The homogenates were centrifuged at 10,000 rpm at 4 °C for 15 min, and the supernatants were collected for enzyme assays. AChE activity was determined colorimetrically according to Ellman et al. (1961). The reaction mixture (final volume = 930 μl) consisted of 25 mM Tris-HCl buffer containing 1 mM $CaCl_2$ (pH = 7.6), 10 μl 20 mM acetylthiocholine iodide (AcSCh), 50 μl 300 μM DTNB, and 20 μl sample. Variation in optical density was measured in duplicate at 410 nm and 25 °C for 1 min using a Jenway 6405 UV–VIS spectrophotometer. Total protein concentration was determined using the Biuret method (Kingbley, 1942), and AChE activity was expressed in nmol of AcSCh hydrolyzed per minute and milligram of protein using a molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1}.

Download English Version:

https://daneshyari.com/en/article/4418967

Download Persian Version:

https://daneshyari.com/article/4418967

Daneshyari.com