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Short Communication

EFFECT OF DICHLORVOS ON THE ACETYLCHOLINESTERASE FROM TAMBAQUI (*COLOSSOMA MACROPOMUM*) BRAIN

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Abstract—Dichlorvos is an acutely toxic organophosphorous pesticide that is known as a classical acetylcholinesterase (AChE; EC 3.1.1.7) inhibitor. Here, the brain AChE from the important Amazonian fish tambaqui (*Colossoma macropomum*) was assayed in the presence of this insecticide and also of deltamethrin, a classical sodium and potassium channel inhibitor (negative control). Four tissue homogenates were analyzed in triplicate for AChE activity using acetylthiocholine as the substrate and 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) as the color-developing agent. Each tissue homogenate represented pooled brains from five fish. The inhibitory effect of dichlorvos on AChE activities was determined at concentrations from 0.001 to 10 ppm and compared to controls. This effect followed an exponential decay model ($y = 9.420 + 26.192e^{(-x/5.380)}$; $r^2 = 0.989$), presenting IC50 (the concentration of dichlorvos that is required for 50% of AChE inhibition) of 0.081 ppm (0.368 μmol/L). No effect was observed for the deltamethrin, and the concentration 0.0452 μmol/L of dichlorvos was significantly different from this control. These results suggest that tambaqui brain AChE can be proposed as a biomarker for dichlorvos and can be used as a tool for aquatic environment monitoring.

Keywords—Organophosphorous pesticide Dichlorvos Biomarker Acetylcholinesterase Tambaqui (*Colossoma macropomum*)

INTRODUCTION

Environmental monitoring is of paramount importance for the management of any area. Several methodologies have been recently developed in order to monitor aquatic environments [1–4], and the use of fish or molecules extracted from them is amongst the in vivo or in vitro detection methods for this objective [3,5,6]. Acetylcholinesterase (AChE; EC: 3.1.1.7) is responsible for degrading acetylcholine in synaptic gaps (cholinergic synapses) and neuromuscular junctions. This enzyme has proved to be a good biomarker for monitoring contaminant concentrations in aquatic environments [7–9]. It has been widely used as a biomarker to detect the occurrence of organophosphorous and carbamate pesticides in the environment [10,11], mainly due to the good correlation observed between the AChE activity and toxic effects [9].

Dichlorvos is an organophosphorous pesticide widely used to combat outdoor and in-home mosquito vectors of several tropical diseases, and it is also used in tropical aquaculture to control ectoparasitic infections, generating contamination in aquatic environments. This pesticide is a direct-acting inhibitor of AChE that provokes an accumulation of acetylcholine in synapses with disruption of the nerve function causing parasympathetic disorders and death of the organism [11].

In this context, fish and their enzymes are good biomarkers and have widely been employed for environmental monitoring [2,4,8,10–13]. Here, brain from juveniles of tambaqui (*Colossoma macropomum*), one of the most important Amazonian fish and a native species in Brazilian aquaculture, was proposed as a source of AChE for sensitive dichlorvos monitoring. The use of this fish may represent an important contribution to environmental monitoring and therefore conservation.

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MATERIALS AND METHODS

Materials

Acetylthiocholine iodide, bovine serum albumin, 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB), and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane and HCl were from Merck (Darmstadt, Germany). Dichlorvos (Mafu®; 1.0%) and deltamethrin (Penetrol®; 0.01%) were acquired from Bayer (São Paulo, SP, Brazil) and Otto Baumgart (São Paulo, SP, Brazil), respectively. The juveniles specimens of *C. macropomum* were supplied by Mar Doce Piscicultura e Projetos (Camaragibe-PE, Brazil). Specimens of tambaqui 17.3 ± 3.2 cm in length and 16.5 ± 2.3 g in weight were captured from 600-m² outdoor tanks (dissolved oxygen 6.2 ± 0.3 ppm, temperature 26.5 ± 0.2°C, pH 8.26 ± 0.2) and kept at 4°C during transportation to the laboratory (~30 min).

Methods

Enzyme extraction. Five juvenile fish were sacrificed through immersion during 10 min in an ice bath (0°C) and their brains immediately removed and homogenized to a final concentration of 40 mg/ml in 0.1 mol/L Tris-HCl buffer, pH 8.0. Afterwards, this homogenate was centrifuged for 10 min at 5,000 *g* (4°C) to remove cell debris. The supernatants, from now on called crude extract, were frozen at −20°C (not exceeding a storage time of 48 h) and then used for further assays [8,14].

Protein determination. Protein content was estimated ac-

cording to a modified dye binding method [15] using bovine serum albumin as standard protein.

Enzyme activity. The crude extract (60 μl) was added to 1 ml of 0.25 mM DTNB (dissolved in 0.5 mol/L Tris-HCl buffer, pH 7.4) and the reaction started by the addition of 0.125 mol/L acetylthiocholine iodide (60 μl) [16]. The enzymatic activity (triplicate) was monitored in a spectrophotometer at 405 nm for 3 min during which the reaction followed a first order kinetics pattern. A unit of activity (U) was defined as the amount of enzyme capable of converting 1 μmol of substrate per minute. The blank assay was similarly prepared except that 0.1 mol/L Tris-HCl buffer, pH 7.4, replaced the crude extract sample.

Inhibition assay. The assays for AChE inhibition by two commercially available insecticides were carried out using Mafu and Penetrol in which the active components are dichlorvos (an organophosphate) and deltamethrin (a pyrethroid), respectively. The last one was used as a negative control because it does not have any effect on the AChE activity [17]. The insecticides were first dissolved in DMSO and then diluted in distilled water to a final concentration of 0.001, 0.005, 0.01, 0.1, 1.0, and 10.0 ppm. The insecticide solutions (20 μl) were incubated with the crude extract (40 μl) for 1 h and the residual activity (%) was determined as previously described, using the enzymatic activities in the absence of pesticide as 100%. All the enzymatic and inhibition assays (triplicate) were carried out at room temperature (25°C). Four tissue homogenates pools from five fish brains were analyzed in triplicate for each insecticide concentration and data were expressed as mean ± standard deviation of 12 assays. These data were statistically analyzed and fitted to exponential decay by using the software MicroCal™ Origin™ Version 6.0 (MicroCal, Northampton, MA, USA).

RESULTS AND DISCUSSION

The crude extract of *C. macropomum* brain presented a specific activity of 38.3 mU/mg of protein. This enzyme preparation was exposed to increasing concentrations of dichlorvos and deltamethrin to assess the inhibition of AChE activity. There was no difference ($p > 0.05$) between the AChE activities in the presence and absence of deltamethrin (negative control), which is expected since this pesticide does not have anticholinesterase effects [18]. This result also demonstrates that DMSO, used as a solvent for the deltamethrin and dichlorvos, does not have any effect on the acetylcholinesterase activity from tambaqui brain.

In contrast, dichlorvos was capable of inhibiting AChE extracted from tambaqui even at concentrations as low as 0.005 ppm (0.226 μmol/L) when 18% of inhibition was detected. An exponential decay ($r^2 = 0.989$; $y = 9.420 + 26.192e^{(-x/5.380)}$, where x is the natural log of dichlorvos concentration in parts per million) of activity was obtained when the enzyme activity was measured after incubation with increasing concentrations of dichlorvos (Fig. 1).

The assay herein described demonstrated high sensitivity for the presence of dichlorvos. This sensitivity was confirmed by estimating the IC50 of AChE from this source in presence of this pesticide, which was found to be 0.081 ppm (0.368 μmol/L). The IC50 value reported for the AChE activity from European sea bass (*Dicentrarchus labrax*) was approximately 90-fold higher (7.34 ppm or 30.3 μmol/L) [11,13]. In contrast to the present study, the experiment with European sea bass was carried out using acetone as the dichlorvos solvent [11].

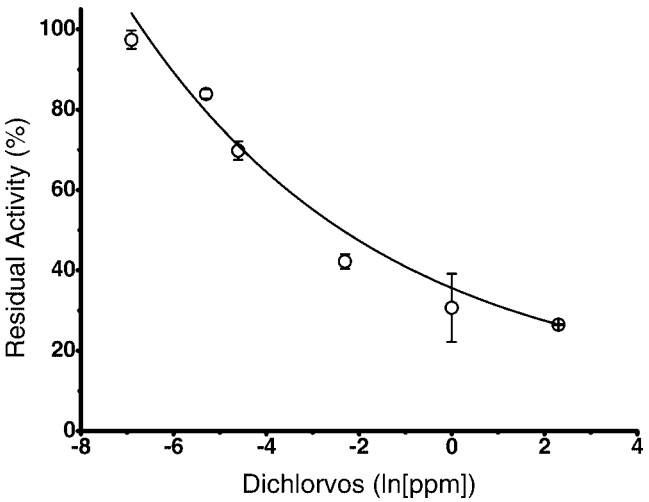

Fig. 1. Effect of increasing concentrations of dichlorvos on acetylcholinesterase (AChE) extracted from brain of juvenile *Colossoma macropomum*. The assay was performed at 25°C as described in the *Materials and Methods* section and the experimental points are the mean ± standard deviation of triplicate of four crude extracts obtained from five brains each ($y = 9.420 + 26.192e^{(-x/5.380)}$; $r^2 = 0.989$).

However, no statistically differences ($p > 0.05$) were found to tambaqui brain AChE inhibition by dichlorvos dissolved either by DMSO or acetone (data not shown).

Although several methodologies have been described for pesticide monitoring in aquatic environment those based on in vitro procedures are by far cheaper, more sensitive, less time-consuming, and less laborious than in vivo assays. Furthermore, the enzyme sensor rather than either tissue or animal sensor directly interacts with the inhibitor, excluding several features that can interfere in the use of latter biomaterials.

According to these results, the enzyme from brain of juvenile tambaqui was shown to be very sensitive and useful as a tool for aquatic environment monitoring.

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