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RESEARCH NOTE/ NOTE CIENTIFICA

EFFECT OF TBT IN ACETYLCHOLINESTERASE OF *PERNA PERNA* (LINNAEUS, 1758) (MYTILIDAE)

EFECTO DE TBT EN ACETILCOLINESTERASA DE *PERNA PERNA* (LINNAEUS 1758) (MYTILIDAE)

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ABSTRACT

Acetylcholinesterase (AChE) is one of the most well-known biomarkers in ecotoxicological monitoring programs of organophosphates and carbamates. However, some studies suggest that acetylcholinesterase also act as a biomarker for other xenobiotics. With this approach we conducted comparison tests of this biomarker in two locations in the city of Arraial do Cabo – RJ, Brazil. Dos Anjos Beach is a cove that has a port considered as an environment impacted by tributyltin, with the oceanic beach Praia Grande introduced in our study as a reference environment. In two samples (03/04/2004 and 09/06/2005) the specific activities of acetylcholinesterase activity in gills of *Pernaperna* were higher in Dos Anjos Beach corroborating laboratory experiments conducted by other authors. Additional studies are important for the determination of imposex associated with acetylcholinesterase, and can also integrate the function of biomarkers for exposure to tributyltin in molluscs.

Keywords:acetylcholinsterase - Arraial do Cabo – biomarker – tributiltin.

RESUMEN

La acetilcolineterasa (AChE) es uno de los biomarcadores más conocidos en los programas de monitoreo ecotoxicológico de los organofosforados y carbamatos. Sin embargo, algunos estudios plantean la posibilidad de que la acetilcolinesterasa también actúe como un biomarcador para otros xenobióticos. Con este enfoque hemos realizado pruebas de comparación de este biomarcador en dos ubicaciones en la ciudad de Arraial do Cabo – RJ, Brasil. La playa dos Anjos, una ensenada que alberga un puerto considerado como un entorno impactado por tributilestaño y una playa oceánica Praia Grande que se introdujo en nuestro estudio como un entorno de referencia. En dos muestras (03/04/2004 y 09/06/2005) las actividades específicas de la actividad de la acetilcolinesterasa en branquias de *P perna* fueron mayores en la playa dos Anjos, corroborando los experimentos de laboratorio realizados por otros autores. Estudios adicionales son importantes para la determinación del imposex asociado a la acetilcolinesterasa, y también puede integrar la función de los biomarcadores de exposición a tributilestaño en los moluscos.

Palabras clave: acetilcolinesterasa - Arraial do Cabo - biomarcador – tributilestaño.

INTRODUCTION

In ecotoxicology, biomarkers is the term applied to any biological alteration showing the exposure or the effect of foreign chemicals (xenobiotics) to the body (Walker *et al.* 2001). The cholinesterases are enzymes that hydrolyze esters (esterase), mainly esters of choline, and acetylcholinesterase (AChE) enzyme involved in neuronal and neuromuscular synapses. The use of these enzymes as biomarkers refers to inhibition by organophosphate and carbamate pesticides (Bocquené *et al.* 1990, Alves *et al*. 2002). It is known that the cholinesterases are also inhibited by a toxin from cyanobacteria, the anatoxin-a (s) (Monserrat *et al.* 2001). Some studies have evaluated the use of cholinesterases as biomarkers generalists or broad spectrum (Payne *et al*. 1996, Guilhermino *et al*. 1998). Whereas other substances related to marine pollution, such as polycyclic aromatic hydrocarbons were also identified as the cause of changes in the specific activity of AChE (Kang & Fang 1997). The relative change in AChE exposure to TBT has been performed in more experiments with fish (Oliveira Ribeiro *et al*. 2002, Greco *et al*. 2007). Few studies have been produced with invertebrate (Puccia *et al.* 2001, Devier *et al.* 2003; Devier *et al*. 2005). Mussels are known biological indicators used in monitoring programs (Salazar & Salazar 1996). Because they are filter feeders, they accumulate different chemicals and have a cosmopolitan distribution that qualifies as a model of sentinel species for the study.

Our research goals are to: Assay the AChE of *Perna perna* (Linnaeus, 1758) gill in environment and investigate the application of acetylcholinesterase as biomarkers of marine pollution caused for TBT

MATERIALS AND METHODS

Study Area

The city of Arraial do Cabo (23 º 00'S, 042 ° 00'W) located 165 km east of Rio de Janeiro, Brazil. The Praia dos Anjos (bay of angels) is located in the inlet of the Angels, where there is a port, a marina and an adjacent pier to the port. The Praia Grande is a beach ocean environment taken as reference, has no apparent activity impacting. Both belong to marine extractive reserve Arraial do Cabo (Figure 1).

Tissue

The gills of *P. perna* were collected in the field and kept on ice until storage at -20 ° C. For each local pools (five individuals, two pools per point) were collected. The length of the sample was measured with a caliper and should present the same average size. The collections of the pool are made in an impacted environment (Praia dos Anjos) and control environment, supposedly without impact (Praia Grande) in 2004 (three samples) and 2005 (one sample).

Processing of tissues and enzymatic assay

In the laboratory the animals are weighed and homogenized with phosphate buffer 0.1 M pH 7.5, the ratio of 1 g wet weight for 10 mL buffer. This preparation is passed at least thirty times in appliance Potter-Elvehjem type (Potter 1955). The enzyme cholinesterase testing is performed in a spectrophotometer by the method of Ellman *et al.* (1961) as adaptation of Lockridge *et al.* (1997). The substrate used was acetylthiocholine iodide at a final concentration of 1.875 mM, DTNB (Ellman's reagent or 5,5'-dithiobis-(2-nitrobenzoic acid)) in the final concentration of 0.32 mM. We used the extinction coefficient of 14,150 M^{-1} cm^{-1} for the quantification of the product formed, tionitrobenzóico acid (TNB). The change in absorbance was measured with a spectrophotometer at 412 nm for 3 min. The

results are presented in miliunits (mU) per gram of wet tissue.

Statistical Analysis

Tests are performed resulting in duplicates with differences of less than 10%. Enzymatic activities are expressed as mean ± standard deviation and t test of Student ($p < 0.05$) using Microsoft Excel ®.

RESULTS

The average length of mussels were: 52±3.86 mm. The gills of *P. perna* collected in two sites of the Arraial do Cabo city State of Rio de Janeiro, Brazil, showed an increase in specific activity of AChE in the environment impacted by port activities (Praia dos Anjos - 03/04/04 and 09/06/05) when compared to Praia Grande (Figure 2).

DISCUSSION

It is well known to the application of acetylcholinesterases as biomarkers of organophosphorus and carbamates pesticides (Alves *et al.* 2002, Oliveira *et al*. 2007). Such use may be extended to a wider biomarking. In our study in two samples (3/04/04 and 9/06/05), where the specific activity at Anjos beach was over the Praia Grande. This fact indicated that induction occurred in the activity of AChE. Some studies corroborate these findings. In these works some aquatic organisms showed increased activity when submitted to certain concentrations of tributyl tin - TBT (Puccia *et al*. 2001, Greco *et al*. 2007). According Puccia *et al.* (2001) study of cholinesterase with a kind of sea squirt - *Ciona intestinalis* (Linnaeus, 1767), the TBT exerts a positive regulation in the expression of this

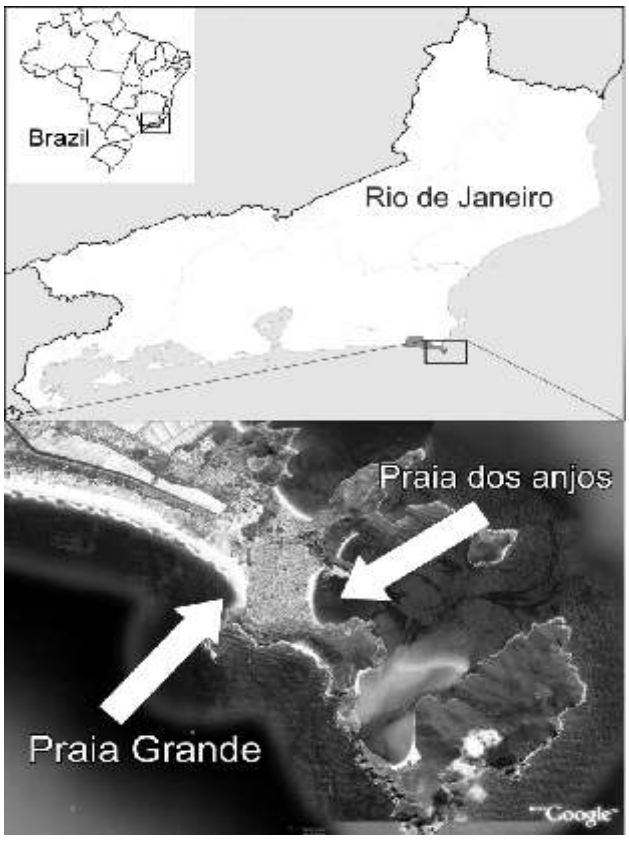

Figure 1. Location of study area.

enzyme by regulating a transcription factor (NF-kB). The species *Salmo salar* (Linnaeus, 1758) showed significant increase in acetylcholinesterase activity in muscles when exposed to concentrations above 250 ng.L^{-1} TBT (Greco *et al*. 2007). Oliveira Ribeiro *et al.* (2002) no observed change in the activity of AChE in muscle *Astyanax bimaculatus* (Linnaeus, 1758) after intraperitoneal doses of TBT.

Beach Angels has a history of contamination by tributyltin due to presence of the port. According to Ribeiro (2002), *Stramonita haemastoma* (Linnaeus, 1767) populations of the bay of angels in Arraial do Cabo suffered from an endocrine disorder called imposex, where females of this species of gastropod had penis and vas deferens. Toste *et al.* (2011) working in the area in question in 2002 and 2008, showed that the beaches Angels region where the port of the furnace suffered impact caused by TBT. Using imposex in *S. haemastoma*, they note that the areas impacted in 2002, no longer possessed this species in 2008. This situation is indicative of contamination by tributyltin.

On the other hand, we cannot rule out the possibility of other metals have caused this effect on AChE. According Bainy *et al.* (2006) Pb and Cd increased specific activity of AChE digestive gland of *P. perna* after 12 and 72 h exposure, respectively. However, the author notes that the induction effect occurred in acute exposure when the exposure was chronic metals began to inhibit AChE.

The highest activity of cholinesterase on the Praia dos Anjos may be related to the presence of compounds inducing expression of AChE, such as tributyltin (TBT). What makes for an important discussion on the use of only the enzyme inhibition as an biomarker of xenobiotics in the environment.

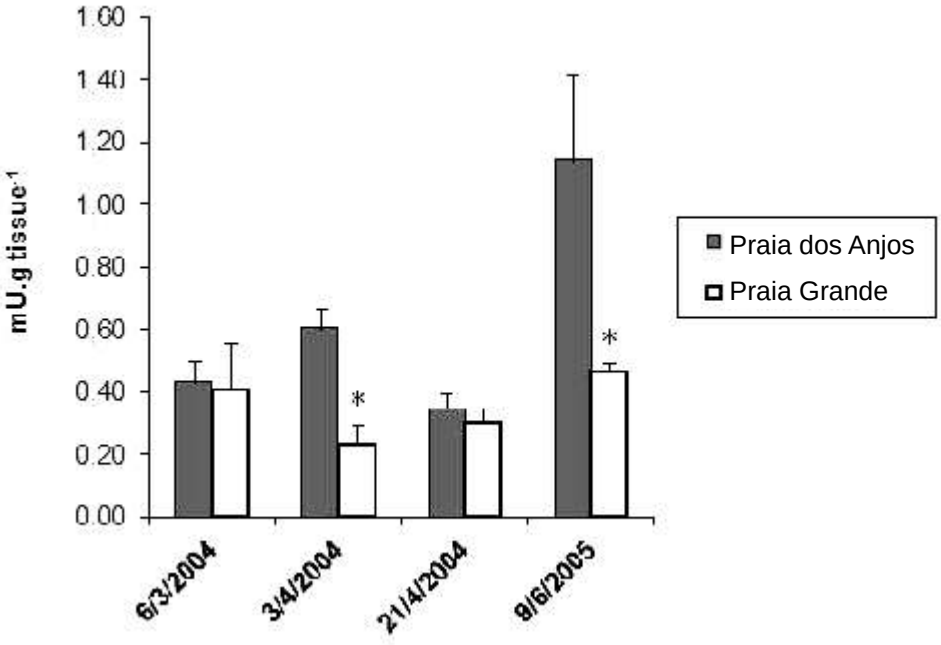

Figure 2. Specific activity of ChE in the gills of *Perna perna* collected in pools in Arraial do Cabo-RJ. Bars are standard deviation of repeatability assay in the sample pool. (n=2 pools)* significant differences.

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