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SHORT COMMUNICATION

Cholinestarase activity in methylmercury and mercury chloride exposure fish

T.B. DE JESUS[1], J.S. COLOMBI[2], C.A.O. RIBEIRO[3], H.C.S. DE ASSIS[4] & C.E.V. DE CARVALHO[2]

[1]Universidade Estadual de Feira de Santana, Programa de Pós-Graduação em Modelagem em Ciências da Terra e do Ambiente, Av. Transnordestina, s/n, Feira de Santana, Bahia, Brasil. Tel: 55- 75- 31618371.

[2]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Laboratório de Ciências Ambientais, Av. Alberto Lamego, 2000, Campos dos Goytacazes-RJ, 28015-602, Brasil. Tel/fax: 55- 22- 27261472.

[3]Laboratório de Toxicologia Celular, Universidade Federal do Paraná. Caixa postal 19031, Centro Politécnico.Bairro Jardim das Américas. CEP 81.531-990. Curitiba – PR Brasil.

[4]Laboratório de Toxicologia Ambiental, Universidade Federal do Paraná. Caixa postal 19031, Centro Politécnico.Bairro Jardim das Américas. CEP 81.531-990. Curitiba – PR Brasil.

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Resumo

No presente estudo, uma injecção intraperitoneal contendo a mesma concentração de MeHg $HgCl_2$ foi administrado em *Hoplias malabaricusa*após 96 horas os peixes foram anestesiados e amostras de tecido muscular foram retirados. Inibição da atividade da colinesterase (CHE) foi observada no músculo do peixe exposto a MeHg, em comparação com o controle. O presente trabalho demonstra que Che é um biomarcador eficaz em peixes expostos através de injecção intraperitoneal com a forma orgânica de mercúrio.

Palavras-chave: Mercúrio, Peixe, Biomarcadores e Acetilcolinesterase

Atividade da Acetilcolinesterase após exposição de peixes a cloreto de mercúrio e metilmercúrio

Abstract

In the current study, an intraperitoneal injection containing the same concentration of MeHg and $HgCl_2$ was administered in *Hoplias malabaricus*, after 96 hours fish were anesthetized and the muscle tissue were removed. A significant inhibition of the ChE was observed the fish exposed to MeHg, compared to control. The present work demonstrates that ChE is an effective biomarker in fish exposed via intraperitoneal injection to the organic forms of mercury.

Key-Words: Mercury, Fish, Biomarkers, Cholinestarase

INTRODUCTION

Cholinesterases are believed to be sensitive to mercury; indeed, exposure of different organisms to sub-lethal concentrations of mercury was shown to induce a significant decrease in cholinesterase activities in several organs (Gill *et al*., 1990; El-Demerdash, 2001). The present study was conducted to determine if the bioaccumulation of Hg by *Hoplias malabaricus* (traíra) could be related a significant decrease in cholinesterase activities.

MATERIALS & METHODS

Hoplias malabaricus specimens were collected between July and October 2008 in lakes located at the Itaocara municipality, northwest of Rio de Janeiro State. The health

*Corresponding author: T. B. de Jesus, e-mail:taisebj@hotmail.com

specimens were placed in aquaria with continuous water flux for seven days to acclimatize. The weight and length (mean and standard deviation) of the specimens used in the experiment 96 hours after the exposure times. The specimens were exposed to the same concentrations of organic (MeHg) and inorganic mercury ($HgCl_2$) (0.75 µg g^{-1}) through intraperitoneal injection. After 96 hours of exposure, the specimens were anesthetized with eugenol (1%) and dissected to obtain the axial muscle tissue used in the biochemical analyses. For the analyses, the muscle samples (200 to 300mg) were unfrozen and homogenized with 1 mL of phosphate buffer (0.1 M, pH 7). The homogenized samples were transferred to 2 mL eppendorfs and centrifuged for 10 minutes at 4ºC at a speed of 10,0000 x g.

The supernatants were used in the determination of the cholinesterase activity, according to the method of Ellmann *et al.* (1961), modified to microplates by Silva de Assis (1998). The method was based on the development of the color reaction between the DTNB color reagent and the thiocolin. Was read in the Tecan Sunshine spectrophotometer at 412 nm and the activity was expressed in $nmol.min^{-1}.mg^{-1}$ of protein. The protein concentration was measured according to Bradford's method (1976), with serum bovine albumin (BSA) as the standard.

The normality and homogeneity of data, assumptions for the use of analysis of variance (ANOVA) parametric methods were tested using the Kolmogorov-Smirnov test (Zar, 1984). When necessary, data were transformed to logarithms to base 10.

RESULTS

Cholinesterase activity

A significant inhibition of cholinesterase activity was observed for specimens exposed to methylmercury in comparison to the control and $HgCl_2$ groups (Fig. 1).

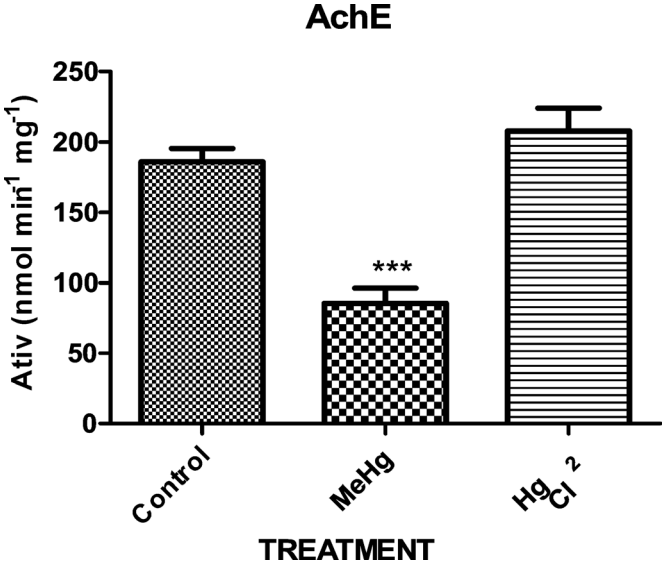

Figure 1.- Cholinesterase activity ($nmol.min^{-1}\ mg^{-1}$) in fish muscle (*Hoplias malabaricus*) after 96 hours of exposure. ***($p < 0.0001$).

Specimens contaminated with $HgCl_2$ presented no significant difference ($p > 0.05$) in the cholinesterase activity, when compared to the control group.

DISCUSSION

In several aquatic animals, AChE and other cholinesterases (ChE) have been found to be inhibited by mercury, in both in vivo and in vitro conditions (Garcia *et al*., 2000; Roméo *et al*.,2006; Elumalai *et al*., 2007). However, no significant effects on AChE (Frasco *et al*., 2008) and increases of AChE activity have been reported (Frasco *et al*., 2007). The results obtained in the present study demonstrated that the inhibition of the cholinesterase activity in muscle of *H. malabaricus* resulted in great susceptibility of this species to methylmercury. In a study by Liao *et.al*. (2006), which used *Oryzias latipes*, a Japanese fish species, the inhibition of acetylcholinesterase in all tissues (liver, brain, muscle and gill) of the specimens exposed to MeHg was verified.

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