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Inhibition of Acetylcholinesterase Activity in the Central Nervous System of the Red Swamp Crayfish, *Procambarus clarkii,* by Mercury, Cadmium, and Lead

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There is need to identify reliable biomarkers to assess the effects of environmental pollution on aquatic organisms. In this regard, the toxicological, physiological and biochemical responses of aquatic crustaceans to heavy metals have been reported by several investigators. Levels of glucose, lactic acid, sodium, potassium, aspartate aminotransferase and alanine aminotransferase in the blood of the crab *Scylla serrata* increased, while glycogen levels in hepatopancreas and muscle decreased after a four–week exposure to mercuric chloride (Kulkarni 1990). In our recent study (Devi *et al.* 1993) with the fiddler crab, *Uca pugilator,* effects of a 48 hr cadmium exposure on lactate dehydrogenase activity were investigated. Enzyme activity was observed to decrease in the hepatopancreas but increased in abdominal muscle. In the red swamp crayfish, *Procambarus clarkii,* exposed for 96 hr to cadmium, glutathione (GSH) level and GSH *S*–transferase activity decreased in the midgut (Almar *et al.* 1987). Meyer *et al.* (1991) exposed the crayfish *Astacus astacus* to sublethal concentrations of lead and cadmium and found that oxidative enzyme (succinic dehydrogenase and NADPH–cytochrome P450 reductase) activities in gills and hepatopancreas decreased. In addition, SH and S–S groups in microscopic sections of these tissues from exposed crayfish exhibited stronger staining reactions than tissues of control crayfish, whereas the staining intensities of cytochrome c oxidase and NADH and NADPH diaphorases in tissue sections from exposed crayfish decreased. Acetylcholinesterase (AChE) inhibition by organophosphates and organocarbamates in various crustaceans was reported by Repetto *et al.* (1988), Surendranath *et al.* (1990) and Ramana Rao *et al.* (1991). *In vivo* cadmium exposure caused increases in esterase activities, but mercury exposure decreases these activities in the hepatopancreas of the shrimp *Callianassa tyrrhena* (Thaker and Haritos 1989a,b). The freshwater field crab, *Barytelphusa guerini*, exposed to 0.6 ppm cadmium showed reduced oxygen consumption throughout the experiment whereas AChE activity increased after 4 days but decreased after 15 days (Reddy and Venugopal 1993).

Our main interest was to determine the effects of cadmium, lead and mercury on AChE activity in central nervous tissue of *Procambarus clarkii* . This enzyme has the potential for serving both as a biochemical indicator of toxic stress and a sensitive parameter for testing water for the presence of toxicants. These three biologically silent metals have, according to Schweinsberg and Karsa (1990), great toxicological significance to humans because their use is widespread.

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MATERIALS AND METHODS

Procambarus clarkii, procured from a local seafood dealer in September and October, 1994, were acclimated to laboratory conditions for 15 days prior to use. Crayfish were maintained at room temperature, 23 ± 2°C, under controlled photoperiods of 12:12 hr light:dark. The water was changed daily and crayfish were fed on commercially prepared crayfish pellets (People's Moss Gin Company, Palmetto, LA). The animals selected for use in the study were starved for 48 hr prior to and during the study. One hundred and twenty adult intact female intermolt crayfish having a body weight 28 ± 4 g were used.

Stock solutions of mercuric chloride (99% pure) (100 ppm), cadmium chloride (99% pure) (100 ppm) and lead nitrate (99% pure) (1000 ppm) (Aldrich Chemical Company) were prepared in deionized water. Lead nitrate was dissolved by the addition of 1 ml of concentrated nitric acid per liter of water. Nitric acid control was used in lead experiments.

For each metal, 20 crayfish were exposed to 4 L of the metal solution and 20 crayfish in 4 L of clean water served as control. Sublethal concentrations of the metals (mercury, 0.2 ppm; cadmium, 5 ppm; lead, 100 ppm) were used in various exposures. For each metal, after 24 hr exposure the AChE activity in the brain and ventral nerve cord of 10 exposed and 10 control animals was measured, and again after 48 hr. The solutions were changed daily to assure that intended concentrations did not change appreciably. The nervous tissues from the control and experimental animals were rapidly isolated and kept on dry ice. The tissues were weighed and homogenized in ice cold 0.1M, 8.0 pH phosphate buffer (1% w/v). The crude homogenate was used as the enzyme source.

AChE (acetylcholine acetylhydrolase, EC 3.1.1.7) activity was assayed by the method of Ellaman *et al.* (1961) with the aid of a Hewlett Packard 8452A Diode Array Spectrophotometer attached to an IBM computer and a Hewlett Packard Recorder. Acetylthiocholine iodide (Sigma) was used as the substrate and 5,5'–dithiobis–2–nitrobenzoate (DTNB) (Sigma) was the thiol indicator. The 3.12–ml reaction mixture contained: 2.95 ml phosphate buffer (pH 8.0, 0.1M), 100 µl 5,5'–dithiobis–2–nitrobenzoate (0.01M in 0.1M phosphate buffer, pH 7.0, 15 mg sodium bicarbonate per 10 ml of solution) and 50 µl tissue homogenate. The reaction was initiated by the addition of 20 µl substrate (75mM, acetylthiocholine iodide) and mixture was incubated at 25° C. The absorbance was measured at 412 nm and recorded every second until the reaction terminated. The enzyme activities were calculated from the linear part of the recording for each sample. The following blank was used for each sample: phosphate buffer + DTNB + enzyme or tissue homogenate to correct for non–AChE–dependent formation of TNB (5–thio–2–nitro–benzoic acid). The absorbance was measured at 412 nm; and when it had stopped increasing, the absorbance was reset to zero and then the substrate was added. The change in absorbance per minute were calculated. The resulting value was divided by the molar extinction coefficient of TNB (1.36×10^4). The specific activity of AChE was calculated using bovine erythrocyte cholinesterase as the standard. Enzyme activity was calculated in terms of units / mg protein. Protein was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Means, standard errors of the mean (SEM) and levels of significance (Student's *t* test) were calculated. A probability value of 0.05 or less between the control and experimental values was considered significant.

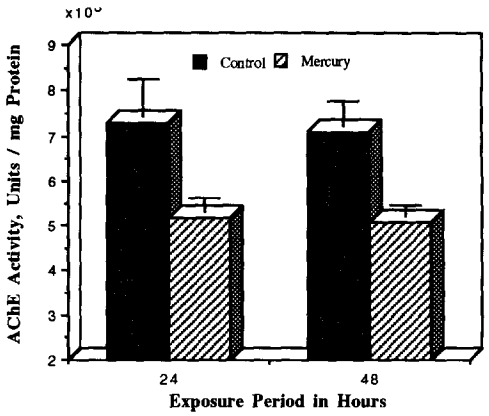

Figure 1. Effect of mercuric chloride on acetylcholinesterase (AChE) activity in central nervous tissue of *Procambarus clarkii* after exposure for 24 and 48 hr. Error bars represent standard error (N= 10).

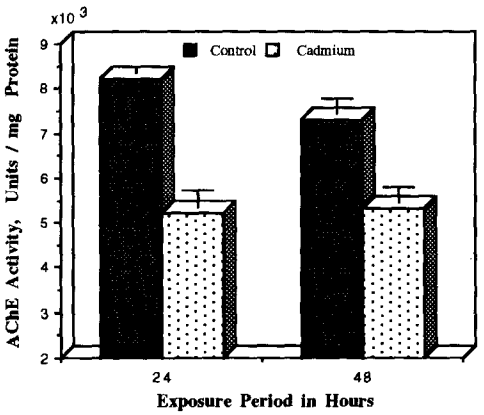

Figure 2. Effect of cadmium chloride on acetylcholinesterase (AChE) activity in central nervous tissue of *Procambarus clarkii* after exposure for 24 and 48 hr. Error bars represent standard error (N= 10).

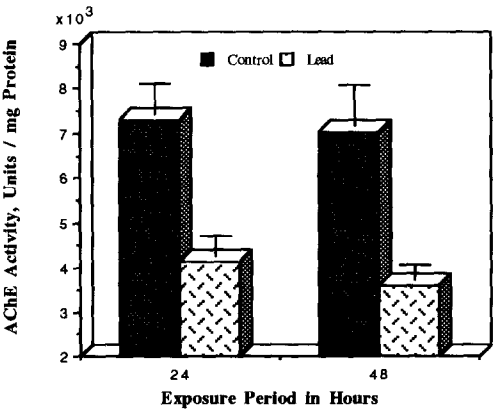

Figure 3. Effect of lead nitrate on acetylcholinesterase (AChE) activity in central nervous tissue of *Procambarus clarkii* after exposure for 24 and 48 hr. Error bars represent standard error (N= 10).

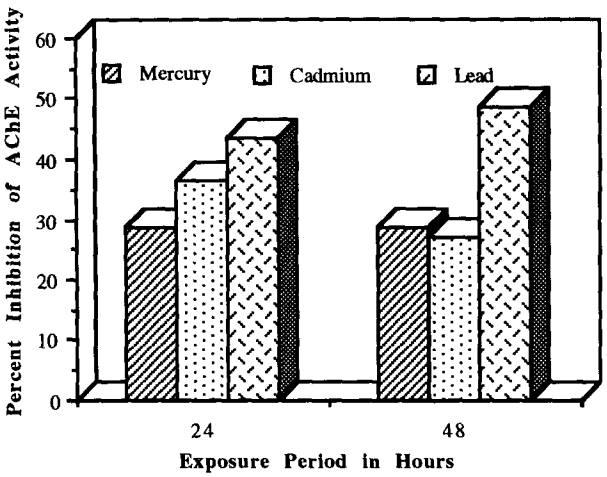

Figure 4. Percent inhibition of acetylcholinesterase (AChE) activity by sublethal concentrations of mercury, cadmium and lead after 24 and 48 hr exposures.

RESULTS AND DISCUSSION

AChE activity decreased significantly after exposure of the crayfish for 24 and 48 hr to mercury (Fig.1), cadmium (Fig. 2) and lead (Fig. 3). In the mercury-exposed crayfish the activity decreased, as compared to control, by 28.81 and 28.66% after 24 and 48 hr respectively (Fig. 4). Similarly, after the 24 and 48 hr exposures cadmium-exposed crayfish showed 36.48 and 27.17% decreases and lead 43.44 and 48.78%.

Inhibition of AChE activity in the central nervous system of the *Procambarus clarkii* exposed to mercury, cadmium or lead is clearly evident in Figures 1-3. These findings concur with those of Thaker and Haritos (1989a) for *Callianassa tyrrhena* hepatopancreas where a 70% decrease in esterase activities was noted after exposure to 0.2 ppm mercury for six days. In contrast, when this shrimp was exposed to 0.1 to 0.8 ppm cadmium, dose–dependent increases in esterase activities in the hepatopancreas were found, which were due to activation of multiple molecular forms of the esterases (Thaker and Haritos 1989b). However, when *Barytelphusa guerini* were exposed to 0.6 ppm of cadmium, AChE activity increased in the thoracic ganglion, muscle and gills after four days of exposure, but then decreased after 15 days of exposure (Reddy and Venugopal 1993).

Mercury, cadmium and lead produce strong inhibition of a large number of enzymes that have functional sulfhydryl groups (Valle and Ulmer 1972). AChE is one such enzyme. However, lead does not inhibit most enzymes that contain a single functional SH group as readily as do mercury and cadmium (Valle and Ulmer 1972). Because of this fact the lead concentration chosen for these exposure studies, while sublethal, was 500 times more than that of the mercury and 20 times more than that of the cadmium. The present paper represents the first report of the effect of metal on AChE activity in a crayfish. Clearly, AChE activity has the potential to serve as a biomarker of heavy metal pollution.

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