Water Temperature as a Source of Variation in Specific Activity of Brain Acetylcholinesterase of Bluegills

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INTRODUCTION

Increased dissemination of highly active pesticides having anticholinesterase activity at very low concentrations has led to greater interest in the enzymatic properties of brain acetylcholinesterase (AChE, EC 3.1.1.7 acetylcholine acetyl-hydrolase). Weiss (1), suggested that inhibition of brain AChE could be used to evaluate the effect of certain pesticides on fish and other aquatic animals. Williams and Sova (2) and Holland et al. (3) reported AChE inhibition in brains of three species of marine fish and one euryhaline species suspected of being exposed to anticholinesterase agents. However, we find there is controversy concerning the influence of different variables on AChE activity, i.e., freezing and storage of samples, environmental temperatures, species, etc.

Weiss (4,5,6) reported that only 40% inhibition of AChE activity in freshwater fish brain may be lethal, and Nicholson (7) suggested that even as little as 10% inhibition could be interpreted as evidence of exposure to anticholinesterase compounds. On the other hand, Gibson et al. (8) reported that freshwater fish experiencing over 90% inhibition of brain AChE activity after being exposed to organophosphorus compounds failed to develop pronounced symptoms of poisoning and recovered completely when placed in fresh water.

The apparent disparity in interpretation of data might be partially explained by the report of Gibson et al. (8) on some possible sources of error in use of brain AChE activity for biologically detecting the presence of AChE inhibiting compounds. A possible source of error not considered by Gibson et al. (8) is the effect of water temperature on the specific activity of AChE from fish brain. Baslow and Nigrelli (9) and Hazel (10) have reported on the possible correlation of AChE activity with water temperature, however, their results are somewhat contradictory. Because both of these studies were accomplished in the laboratory where conditions can be rigidly controlled they might not reflect what occurs under more natural conditions. We decided, therefore,

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 4, 1970, published by Springer-Verlag New York, Inc.

to report on the influence that natural fluctuations in ambient water temperature have on the specific activity of AChE from the brains of bluegills, Lepomis macrochirus.

MATERIALS AND METHODS

Fish: Sexually mature bluegills were obtained from National Fish Hatcheries at Corning, Arkansas, and Guttenberg, Iowa. The fish, about 15 cm long and having an average weight of 150 g, were held in 0.1 ha ponds having an average depth of 0.8 m for at least 30 days prior to analysis. A sample, consisting of 18 fish, was collected once each month for a period of 14 months. The sex was determined by examining the excised gonad. The brains were excised and each brain was analyzed for AChE activity.

Water Temperature: The mean daily temperature was calculated by averaging the minimum and maximum temperatures recorded by two recording thermometers immersed to a depth of 0.1 m or 1 m in a representative pond. All daily readings for the 30-day period prior to the sampling day were used to calculate the mean monthly water temperature.

Air Temperature: The average daily air temperature was obtained from Local Climatological Data reports prepared by the U. S. Department of Commerce. All daily readings for the 30-day period prior to the sampling day were used to calculate the mean monthly air temperature.

Enzyme Preparation: Brain homogenates were prepared as outlined by Hogan and Knowles (11).

Acetylcholinesterase Assay: AChE activity was estimated using a manometric procedure identical to that employed by Knowles and Casida (12). Acetylcholine iodide, 10 mM, served for the substrate. Activity is expressed as μmoles of acetylcholine hydrolyzed per milligram of protein per hour. Duplicate determinations were made on each homogenate.

Protein Determination: Total protein for each homogenate was determined by the Folin-Ciocalteau method (13) as modified by Lowry et al. (14) and Miller (15); fresh bovine serum albumin was used as the standard.

RESULTS

Brain AChE activity was estimated in 93 female and 141 male bluegills. Male and female AChE levels averaged 15.03 ± 2.44 (SD) and 15.79 ± 2.80 umoles/mg per hour, respectively. The mean brain

weights for males was 130.16 ± 22.81 mg and that for females was 125.37 ± 21.37 mg. The differences in AChE activities and brain weights were not statistically significant ($P>0.05$) according to Fisher's "t" test. Therefore, data for both sexes were combined for further statistical analysis.

Graphical comparison of water temperature and air temperature with brain AChE activities for each month suggested a probable correlation (Figure 1). Water temperatures were not available for May and December, 1968, and AChE activity was not estimated in January, 1969. Water and air temperatures, however, appeared to be closely correlated (Figure 1). Therefore, water temperatures were estimated for May and December by extrapolation from air temperatures. By using these estimates, 13 data points, rather than 11, became available for subsequent statistical analysis.

Analysis of variance demonstrated a significant correlation between mean AChE activity and water or air temperature (Table 1). Linear, binomial, quadratic, and exponential regression equations were examined. The variance ratio, F 0.01(11,11), must exceed 4.46 for significant correlations. Variance analysis of binomial, quadratic, linear, and exponential equations correlating bluegill brain AChE with water temperature gave F (11,11) values from 29.3 to 79.1, respectively. Air temperature could only be correlated with AChE activity by a quadratic equation F (11,11)=20.6. The

TABLE 1

AChE activity predicted as a function of water or air temperature

Regression equation	Coefficient of determination R^2	F*
$\text{Log(AChE)}=2.45392 + 0.01650(T_{H_2O})$	0.88	79.1
$\text{AChE}=11.35540 + 0.24806(T_{H_2O})$	0.84	59.3
$" =12.58299 + 0.00829(T_{H_2O})^2$	0.84	55.8
$" =11.82599 + 0.14360(T_{H_2O}) + 0.00363(T_{H_2O})^2$	0.85	29.3
$" =11.29231 + 0.39754(T_{AIR}) + 0.00594(T_{AIR})^2$	0.80	20.6

*F 0.01(11,11)=4.46

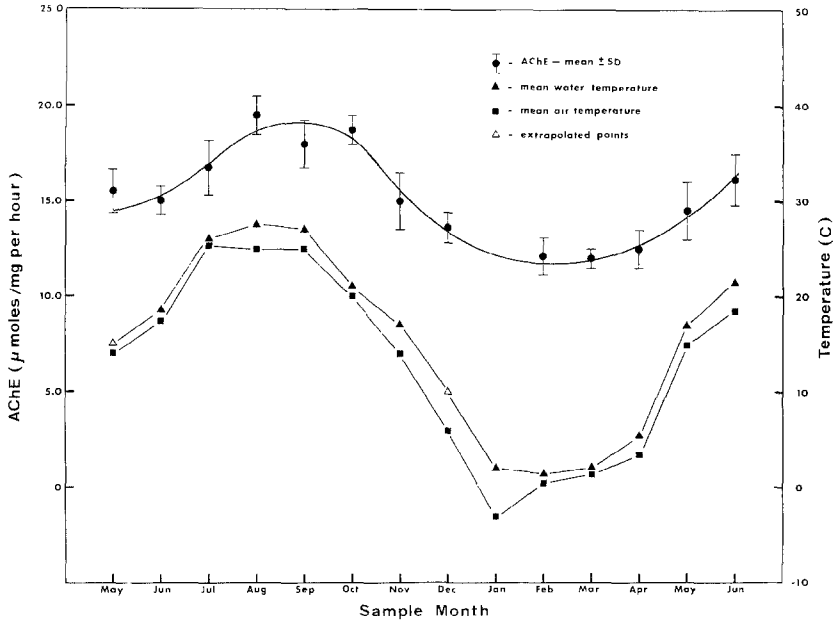

Figure 1. Comparison of water temperature and air temperature with brain AChE activity for each month.

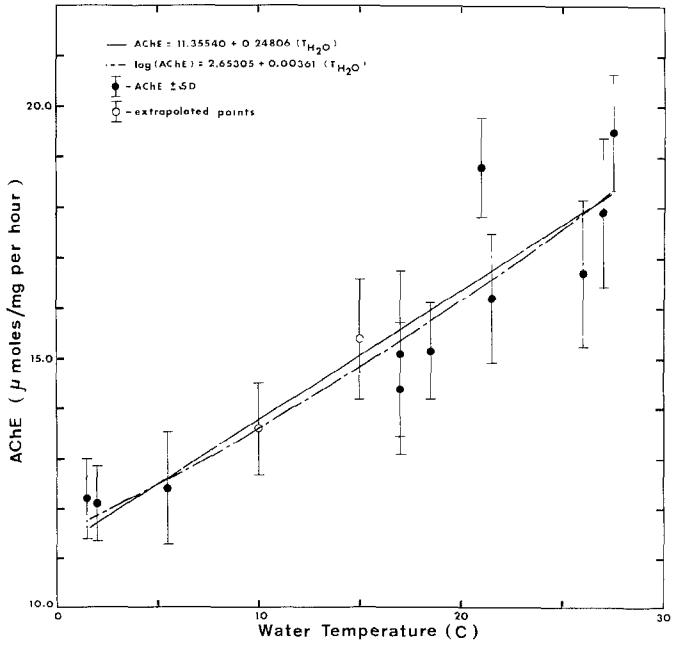

Figure 2. Linear and exponential regression curves of AChE activity with water temperature.

two regression equations giving the most significant correlation between bluegill brain AChE and water temperature were the exponential and the linear equation (Figure 2). In addition, linear regressions were calculated for brain weight-AChE activity and brain weight-water temperature. Neither of these regressions give a statistically significant degree of correlation ($P>0.05$). As a final step, the data were analyzed using the least significant difference (LSD) test (16) to determine whether mean AChE activities for consecutive months were significantly different. In 9 of the 12 possible comparisons the difference was statistically significant ($LSD_{0.05}$).

DISCUSSION AND CONCLUSIONS

Sex does not appear to be an important variable in the use of brain AChE to assess pollution due to anticholinesterase agents, at least when bluegills are the indicator organisms. However, AChE activity of this species varies directly with the environmental water temperature. This finding does not support similar research reported by Baslow and Nigrelli (9) for the killifish, Fundulus heteroclitus. They found that in killifish, the brain cholinesterase (ChE) activity varied inversely with the temperature of acclimation. Also, given a period of acclimation, the changes in brain ChE activity in the killifish occurred in such a way that a specific activity level is maintained regardless of ambient temperature. I obtained no evidence of such a control mechanism in bluegills.

The apparent inconsistencies in temperature-enzyme activities in different fishes may be due to the temperatures at which analyses were conducted, or the methods by which the fish were acclimated to various temperatures. For instance, Baslow and Nigrelli (9) subjected killifish to an eight hour thermal stress period during which the ambient temperature was raised or lowered as much as 18 or 12 C, respectively. By contrast, the fish used in my investigation were subjected to considerably less extreme, and more natural temperature changes over extended periods.

Baslow and Nigrelli (9) analyzed brain ChE in killifish at 26 C, whereas my measurements were made at 37.5 C. In vitro temperature optima for enzymatic activity may correlate with ambient temperature. However, Ludtke and Ohnesorge (17) reported that a correlation did not occur for the tench, Tinca vulgaris. They indicated that no change of temperature-dependent characteristics of the ChE resulted from adaptation to water temperature of 5 to 20 C, and that the optimum assay temperature for tench brain ChE was 34.5 C.

Hazel (10) has recently reported that the specific activity of AChE in brains of both goldfish, Carassius auratus, and killifish acclimated to 25 C was significantly higher than in fish acclimated to 5 or 15 C. In addition, maximum activity was obtained at 35 C and was independent of acclimation temperature for goldfish. In general, my results with bluegills are similar and tend to confirm the observation of Hazel (10) that an increase in ambient temperature results in an increase of AChE specific activity.

In summary, changes in ambient water temperature may be a source of considerable error in the use of bluegill brain AChE for the biological assessment of pollution by anticholinesterase agents.

ACKNOWLEDGEMENTS

The technical assistance of Brenda Yamnitz and Ginger Gibson is gratefully appreciated, as is the advice of Dr. E. S. Hildebrand, Statistician, Agriculture Experiment Station, University of Missouri.

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