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BRAIN CHOLINESTERASE ACTIVITY OF NESTLING GREAT EGRETS, SNOWY EGRETS AND BLACK-CROWNED NIGHT-HERONS

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ABSTRACT: Inhibition of brain cholinesterase (ChE) activity in birds is often used to diagnose exposure or death from organophosphorus or carbamate pesticides. Brain ChE activity in the young of altricial species increases with age; however, this relationship has only been demonstrated in the European starling (*Sturnus vulgaris*). Brain ChE activity of nestling great egrets (*Casmerodius albus*) collected from a colony in Texas (USA) increased significantly with age and did not differ among individuals from different nests. Brain ChE activity of nestling snowy egrets (*Egretta thula*) and black-crowned night-herons (*Nycticorax nycticorax*) collected in one colony each from Rhode Island, Texas and California (USA) also increased significantly with age and did not differ among individuals from different nests or colonies. This study further demonstrates that age must be considered when evaluating exposure of nestling altricial birds to ChE inhibitors.

Key words: Brain cholinesterase activity, black-crowned night-herons, *Nycticorax nycticorax*, great egrets, *Casmerodius albus*, snowy egrets, *Egretta thula*, pesticide monitoring, field study.

INTRODUCTION

The National Contaminant Biomonitoring Program (NCBP) (U.S. Fish and Wildlife Service, Washington, D.C. 20240, USA) includes sampling several vertebrate species for contaminant analysis: freshwater fish, European starlings (*Sturnus vulgaris*) and wings of hunter-killed mallards (*Anas platyrhynchos*) and American black ducks (*Anus rubripes*) (Jacknow et al., 1986). In an effort to include an estuarine component in the NCBP, the U.S. Fish and Wildlife Service is evaluating the possibility of monitoring contaminant exposure in nestling colonial waterbirds.

Detecting exposure to organophosphorus or carbamate pesticides is one type of contaminant monitoring being evaluated for colonial waterbirds. Measurement of brain cholinesterase (ChE) activity is frequently used as an indication of exposure to these anti-ChE chemicals (Hill and Fleming, 1982). An approach to using brain ChE activity for diagnosis of anti-ChE exposure has been suggested by Ludke et al. (1975). Brain ChE activity >20% below controls or two standard deviations below average of controls indicates probable exposure to a ChE inhibitor, and mortality is usually associated with brain ChE activities >50% below controls.

Previous research suggests that the development of brain ChE activity differs between altricial and precocial or semi-precocial avian species. Brain ChE activity increases during embryonic development in precocial or semi-precocial species (Hoffman and Eastin, 1981) and activity is similar for immature and adult birds (Ludke et al., 1975; White et al., 1979). In contrast, brain ChE activity for the altricial European starling is age-dependent (Grue et al., 1981; Grue and Hunter, 1984; Robinson et al., 1988). This phenomenon has not been investigated in other altricial species. The objectives of this study were to determine brain ChE activity of nestling great egrets (*Casmerodius albus*), snowy egrets (*Egretta thula*) and black-crowned night-herons (*Nycticorax nycticorax*); and to deterine if brain ChE activity of these altricial species is age-dependent.

MATERIALS AND METHODS

Herons and egrets were collected in 1987 from one colony each along the Rhode Island, Texas and California coasts. The Rhode Island colony was located on Gould Island in Newport County (Colony number 352003; 41°37'N, 71°13'W; Os-

TABLE 1. *P* values generated from analysis of variance of the effects of nest, colony and age[a] on brain cholinesterase in three colonial waterbird species.

Source of variation	*P* values		
	Great egret	Snowy egret	Black-crowned night-heron
Nest within colony	0.91	0.69	0.91
Colony[b]	—	0.66	0.99
Age	<0.01	<0.01	<0.01
Colony × age	—	0.34	0.73

[a] Age was divided into three categories: 4 to 7, 8 to 12, and 13 to 17 days of age.

[b] Colonies included one each from California, Rhode Island and Texas.

born and Custer, 1978); the Texas colony was located on Goat Island, Harris County (29°45'N, 95°35'W); and the California colony was located on West Marin Island in Marin County, California (37°58'N, 122°28'W).

Complete three, four, or five-egg clutches of snowy egrets and black-crowned night-herons were located in each of the three colonies and complete four-egg clutches of great egrets were located in the Texas colony. In Texas and Rhode Island, nests were examined every 2 to 5 days from early incubation through 15 days after the first egg hatched. In California, nestlings were marked at hatching and followed at 2 to 3 day intervals for 15 days. Day of hatch was determined by observing hatching or estimated by assuming cracking of the eggshell occurred 3 days before hatch and pipping 1 day before hatch (T. W. Custer, pers. obs.) or by body measurements including weight (g) and/or length (mm) of tarsus, forearm or bill of newly found nestlings (T. W. Custer, pers. obs.). Nestlings were individually marked with bands, ink or nail polish as soon as they were discovered. Within each clutch sampled, the nestling from the first egg to hatch was collected when about 15-days-old, the second to hatch was collected when about 10-day-old, and the third to hatch was collected when about 5-day-old. If it was not possible to collect three nestlings from a clutch, the priority of collection was 15-, 10- and then 5-day-old nestlings. The actual ages of nestlings collected were assigned to three categories: 4 to 7, 8 to 12 or 13 to 17 days of age. In addition to nestlings, eggs of snowy egrets were collected at the Texas colony 1 or 3 days prior to hatching.

Nestlings and embryos within eggs were killed by CO_2 gas asphyxiation within 2 hr of collection. The carcasses and eggs were preserved immediately on wet ice and frozen at −20 C within 4 hr of death. The ChE assay was conducted

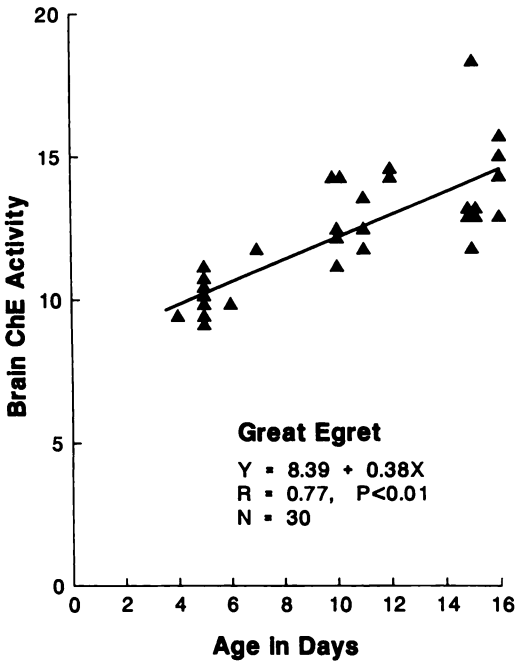

FIGURE 1. Brain ChE activity (μmoles acetylthiocholine iodide hydrolyzed/min/g of tissue, wet weight) of great egret embryos and nestlings.

within 5 months on longitudinally bisected half-brains using the method described by Ellman et al. (1961) and modifications by Hill and Fleming (1982). The reaction was measured on Bausch & Lomb Spectronic 70 spectrophotometer (Bausch & Lomb Inc., Rochester, New York 14604, USA). Acetylthiocholine iodide was used as a substrate. Each brain sample was analyzed in duplicate and an average brain ChE activity was calculated. All samples for each species were analyzed at room temperature (21 to 25 C) on the same day at the Gulf Coast Research Station (Victoria, Texas 77902, USA). Brain ChE activity was expressed as μmoles acetylthiocholine iodide hydrolyzed/min/g of tissue (wet weight).

To determine if colony location, nest or age influenced brain ChE activity of snowy egrets, black-crowned night-herons and great egrets (nest and age only), a two- or three-way ANOVA was conducted. The relationship between nestling age and brain ChE activity was determined using linear and polynomial regression (Snedecor and Cochran, 1967). A *t*-test was used to compare slopes of nestling age and brain ChE activity among species. The level of significance for all statistical tests was $\alpha = 0.05$.

RESULTS

Brain ChE activity of great egrets, snowy egrets, and black-crowned night-herons

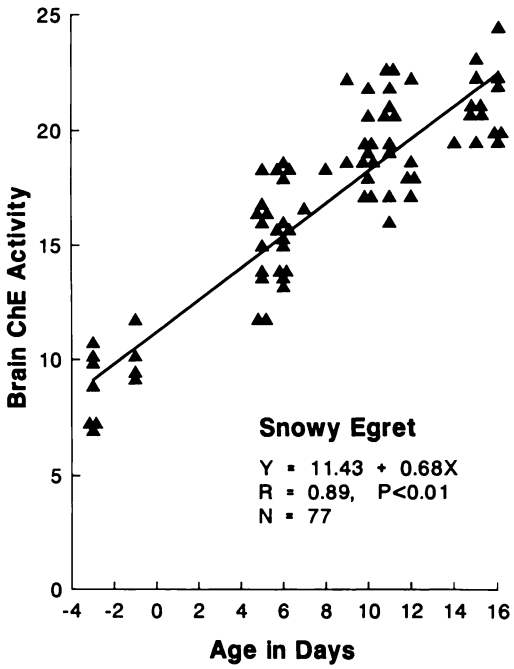

FIGURE 2. Brain ChE activity (μmoles acetylthiocholine iodide hydrolyzed/min/g of tissue, wet weight) of snowy egret nestlings.

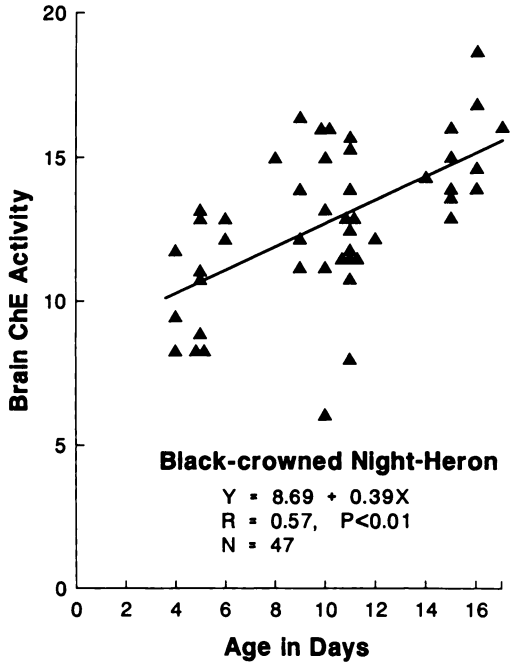

FIGURE 3. Brain ChE activity (μmoles acetylthiocholine iodide hydrolyzed/min/g of tissue, wet weight) of black-crowned night-heron nestlings.

differed significantly ($P < 0.05$) among age categories but did not differ significantly ($P > 0.05$) among colonies (great egrets not included) or among nests (Table 1). Since nest or colony effects were not observed, the data were combined. Brain ChE activity and nestling age were positively correlated and increased linearly for each species (Figs. 1, 2, 3). Polynomial regression was not significantly ($P > 0.05$) different for the great egret or black-crowned night-heron regressions. Polynomial regression was significant ($P < 0.05$) for the snowy egret regression for all data ($Y = 11.234 + 0.91x - 0.02x^2$, $r = 0.90$, $n = 77$) and for nestlings only ($n = 66$); however, because r was only increased by 1 and 2% and in order to make the results comparable to the other species, we use linear regression for presentation in Figure 1 and for comparison with other species. Snowy egret brain ChE activity increased more rapidly ($P < 0.05$) with age (0.68 μmoles acetylthiocholine iodide hydrolyzed/g brain tissue/min/day of age for all data; 0.58 μmoles/g/day for nestlings only) than great egrets (0.38 μmoles/g/day) or black-crowned night-herons (0.40 μmoles/g/day); however, there was no difference ($P > 0.05$) between great egrets and black-crowned night-herons.

Two black-crowned night-heron nestlings had unusually low brain ChE values (Fig. 3). One 11-day-old bird from California had 7.9 μmoles/g and was 40% lower than the value predicted by the regression (13.1 μmoles/g). A 10-day-old nestling from Texas had 6.0 μmoles/g and was 53% lower than the predicted value (12.7 μmoles/g). These values were included in the regression of brain ChE activity versus age (Fig. 3).

DISCUSSION

Nestlings of three altricial waterbird species studied demonstrated increased brain ChE activity with age. These results are similar to those described for starlings by Grue et al. (1981) and Grue and Hunter (1984) which suggest that age must be con-

sidered in evaluating exposure of nestling altricial birds to ChE inhibitors.

This study demonstrates the feasibility of measuring brain ChE activity as part of a National Contaminant Biomonitoring Program using nestling colonial waterbirds. Neither colony location nor nest had a significant effect on nestling brain ChE activity. These results are similar to those of Grue et al. (1981) that demonstrated nest to nest variation did not have a significant effect on brain ChE activity in developing starlings. Although nestling brain ChE activity is age dependent, age can be determined and the relationship between age and brain ChE activity calculated. Age of nestlings can be determined by repeated visits to nests; however, a combination of weight and body measurements may give similar results (T. W. Custer, unpubl. data) and would be less time consuming and would minimize disturbance in the colony.

Brain ChE activity in snowy egrets increased more rapidly with age than great egrets or black-crowned night-herons. This also seemed to be associated with differences in mobility of the nestlings. Fifteen-day-old black-crowned night-herons or great egrets usually remained at the nest when disturbed, but 15-day-old snowy egrets often left the nest when disturbed (T. W. Custer, pers. obs.).

As mentioned elsewhere (Grue and Hunter, 1984), brain ChE levels presented here should not be used as normal values in subsequent studies because the measurement techniques are not standardized. However, brain ChE activity can be used as an indicator of exposure to ChE-inhibitors if proper controls are collected and concurrently analyzed (Ludke et al., 1975). Also, measurements of brain ChE activity from field-collected control specimens (within species) are reproducible provided that consistent assay procedures are followed (Hill, 1988).

A relationship between brain ChE activity and nestling age was demonstrated using data from field-collected birds. The correlation coefficients for brain ChE activity and nestling age for snowy egrets ($r = 0.89$), great egrets ($r = 0.77$) and black-crowned night-herons ($r = 0.57$) were statistically significant but not as high as observed by Grue et al. (1981) for wild starlings ($r = 0.93$). Additional variance in these field-collected data could have occurred because of errors in aging the nestlings, food or heat stress (Rattner, 1982), or potential exposure to ChE-inhibiting chemicals. The variance surrounding all age estimates is unknown, but probably was within 2 days. Food and/or heat stress has been shown to reduce brain ChE activity by 10 to 17% (Rattner, 1982). None of the three colonies was located within major agricultural areas; however, local exposure to ChE-inhibiting chemicals was possible from other sources such as chemicals used to control mosquitoes.

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