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CHANGES IN BRAIN AMINE CONCENTRATIONS ASSOCIATED WITH POSTEMBRYONIC DEVELOPMENT IN THE SOLPUGID, *EREMOBATES PALPISETULOSUS* FICHTER (SOLPUGIDA: EREMOBATIDAE)

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ABSTRACT. Experiments were conducted to study changes in brain amine concentrations during postembryonic development in the solpugid, *Eremobates palpisetulosus*. Data presented here indicate that 5-hydroxytryptamine (5-HT) and dopamine (DA) levels increase during development whereas N-acetyldopamine (NADA) levels were found to decrease. 5-HT levels showed a significant increase between the first and second nymphal instars whereas DA increased significantly after the second nymphal instar stage. Octopamine levels did not change as a function of development in this solpugid. These represent the first data available on changes in brain amines as a function of development for any arachnid. It is suggested that such neurochemical changes may play a role in the regulation of shifts in behavior associated with specific developmental stages.

One of the most intriguing questions in neuroscience pertains to the relationship between brain chemistry and behavior (see reviews by Hokfelt et al. 1984; Punzo 1985; Black et al. 1987). Most of the research on invertebrates has focused on arthropods. Changes in neurochemistry have been associated with a wide variety of specific behaviors in this taxonomic group. For example, significant increases in brain RNA and protein synthesis were found to accompany avoidance learning in theraphosid spiders (Punzo 1988), decapod crustaceans (Punzo 1983) and several insects (Goldsmith et al. 1978; Punzo 1980; Lin & Roelofs 1992). Inhibition of brain protein synthesis was found to impair learning and memory in insects (Brown & Noble 1967; Jaffe 1980; Punzo 1980) as well as innate phototactic behavior in tenebrionid and passalid beetles (Punzo & Jellies 1980). Biogenic amines (catecholamines and indolamines) have been implicated in a variety of ontogenetic behavioral changes in honeybee workers including shifts in discrimination between olfactory cues (Macmillan & Mercer 1987) and the onset of guarding behavior (Moore et al. 1987). Brain dopamine (DA) levels were significantly higher in forager honeybees than in nurses or food storers (Mercer et al. 1983) suggesting an age-dependent shift in brain amine concentrations. Changes in brain catecholamine levels have also been associated with learning and memory in insects (Mercer & Menzel 1982; Bicker & Menzel, 1989).

Although studies have been conducted on the identification of biogenic amines in the brain of a few spider species (Meyer & Jehnen 1980; Meyer et al. 1984), no attempt has been made to correlate such neurochemical changes with morphological or behavioral changes associated with development. Until recently, no data were available on the neurochemistry of Solpugida (Punzo, in press). Solpugids do exhibit well-defined ontogenetic shifts in behavior (Lawrence 1947; Cloudsley-Thompson 1977). Newly hatched postembryos are gregarious, do not feed and are essentially immobile. First-instar nymphs resemble adults in general appearance and increase their locomotor activity. During the second nymphal instar, solpugids begin to hunt, feed, burrow, and they become increasingly agonistic toward conspecifics. In the present study, I have identified changes in brain amine levels which accompany postembryonic development in the solpugid, *Eremobates palpisetulosus* Fichter. This represents the first analysis of ontogenetic changes in brain amines for any arachnid. This type of information is essential if we are to fully understand the functional significance of differences in brain chemistry and the role (if any) of biogenic amines in the onset of behavioral responses associated with specific developmental stages.

METHODS

Subjects.—Solpugids used in this study were obtained as hatchlings from eggs deposited in the laboratory by gravid females collected within a 3 km radius of Terlingua, Texas (Brewster County) during June–July, 1990. Gravid females were housed individually in plastic containers (30 × 15 × 10 cm) and fed on a diet of mealworm larvae, crickets and grasshoppers as described by Punzo (in press). Each container was provided with a sandy substrate which allowed females to excavate burrows. Following oviposition, eggs were removed and transferred to glass bowls and maintained at 28 °C and 72% relative humidity until hatching occurred. Individual solpugids were randomly assigned to different groups and allowed to mature to various developmental stages. The developmental stages chosen for chemical analysis were based on those described by Muma (1966) and Punzo (in press): post-embryos (PE, hatchlings), first-instar nymphs (N_1, 8 days after hatching), second-instar nymphs (N_2, 17 days after hatching) and adult females (A, 92 days after hatching). All instars were verified by direct observations on molting.

Experimental procedure.—Twenty head capsules of individuals from each developmental stage were removed for analyses. Brains were dissected from the head capsules as described by Punzo & Malatesta (1988). All glandular and peripheral fatty tissues, as well as retinal pigment from the optic lobes, were carefully removed from the surface of brain samples as reported by Murdock & Omar (1981). Individual brains were weighed in a Sartorius Model 54C electronic analytical balance.

Individual brains were tested for the presence of 5-hydroxytryptamine (5-HT, serotonin), octopamine (OA), dopamine (DA) and N-acetyldopamine (NADA) using high performance liquid chromatography with electrochemical detection (HPLC-ED, Beckman Model 47A) as described by Brandes et al. (1990). To summarize, each brain sample was placed in a 500 μl glass vial. Fifty μl of 200 mM perchloric acid were added and the sample homogenized. Following homogenization, an additional 50 μl of perchloric acid were added to each vial. Samples were then centrifuged at 10,000 *g* for 3 min in a Sorvall Model 100A high speed centrifuge. Twenty μl of supernatant were injected directly into the HPLC column (40 cm in length, with a 0.2 μ pore diameter) packed with Hypersil and provided with a Hewlett-Packard 760E detector (0.40 V). The mobile phase (flow rate, 3000 psi) used to elute the biogenic amines consisted of 12% acetonitrile, 20 mM sodium acetate, 100 mM sodium dihydrogen orthophosphate, 2.5 mM octane sulfonic acid and 0.3 mM EDTA disodium salt adjusted to pH 4.2 and filtered through a 0.45 μm filter. Each sample was compared to 5-HT and DA standards tested at the beginning of each assay run and retested at 30 min intervals. Amine concentrations were expressed as pmol/mg of brain tissue. The mean amine concentration and SE were calculated for each developmental stage. A one-way ANOVA followed by a Scheffe multiple comparison test (Sokal & Rohlf 1981) was used to test for significant differences between groups.

Table 1.—Concentrations of 5-hydroxytryptamine (5-HT), octopamine (OA), dopamine (DA) and N-acetyldopamine (NADA) in the brain of *Eremobates palpisetulosus* at various developmental stages: post-embryos (PE), first-instar nymphs (N_1) second-instar nymphs (N_2) and adult females (A). Values represent means (±SE). Values followed by different letters are significantly different ($P < 0.05$).

	Mean concentration (pmol/mg) ± (SE)			
Stage	OA	5-HT	DA	NADA
PE	1.9 (0.3) a	0.7 (0.2) a	8.6 (1.3) a	6.1 (1.2) a
N_1	2.1 (0.5) a	1.1 (0.3) a	11.2 (1.6) a	5.6 (0.4) a
N_2	2.3 (0.5) a	2.4 (0.4) b	20.1 (2.8) b	4.8 (0.5) a
A	2.1 (0.4) a	3.1 (0.7) b	21.3 (2.4) b	2.2 (0.3) b

RESULTS AND DISCUSSION

Concentrations of brain amines at various developmental stages of *E. palpisetulosus* are shown in Table 1. Octopamine levels remained constant during maturation. Serotonin (5-HT) and DA levels increase during postembryonic development whereas NADA levels were found to decrease. Serotonin levels show a significant increase between nymphal instars 1 and 2 whereas DA levels increase significantly after the N_2 stage of development. NADA levels decrease significantly after the N_2 stage. Thus, lower DA and 5-HT concentrations are associated with those developmental stages characterized by low levels of locomotor activity and the absence of aggressive behavior. Agonistic interactions between siblings and other conspecifics first appear in second-instar nymphs (Cloudsley-Thompson 1977; pers. obs.) which also exhibit significantly higher concentrations of brain 5-HT and DA. It is also during this stage that solpugids begin to burrow and actively hunt for food. A previous study showed that acetylcholine (ACh) and acetylcholinesterase (AChE) levels also increase between the first and second instars as well as other developmental stages in solpugids (Punzo, in press).

Changes in brain amine concentrations have been found to accompany morphological and behavioral changes in insects. In insects such as honeybees, ants and flies, significant increases in catecholamines are associated with morphological changes in the transition from larval to pupal stages (Evans 1980; Macmillan & Mercer 1987; Brandes et al. 1990). An increase in serotonin levels has been found to accompany adult emergence in dipteran and social insects (Dewhurst et al. 1972; Brown & Nestler 1985). It is also well known that a reversal in geotactic behavior accompanies the transition from larval to adult stages in many dipteran species and molecular events have been implicated in this reversal (Quinn 1984). Although octopamine levels did not change significantly as a function of maturation in *E. palpisetulosus*, increases in OA levels have been reported for insects (Michelson 1988; Bicker & Menzel 1989). This amine has been implicated in the regulaton of responsiveness to olfactory and visual stimuli due to the fact that concentrations have been found to increase in the antennal and optic lobes of the brain during postembryonic development. With respect to arachnids, Meyer et al. (1984) have shown that there are differences in the brain weight/body weight ratios as well as putative neurotransmitter levels of active wandering spiders such as salticids as compared to araneids and theraphosids. The data from this study suggest that solpugids also lend themselves to studies designed to explore the functional role of biogenic amines in the regulation of behavior. Results also indicate that some brain amine levels undergo significant changes during development and may play a role in regulating the changes that occur in the behavioral repertoire of this solpugid as development proceeds. Further studies are needed to correlate these neurochemical changes with specific morphological changes occurring within the brain during postembryonic development. Future studies should also identify those specific brain regions in which most of these neurochemical events are occurring.

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