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HISTOCHEMICAL LOCALIZATION OF ACETYLCHOLINESTERASE ACTIVITY IN NEURAL TISSUES OF THE GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* DE MAN

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ABSTRACT

The optic lobe, optic peduncle, brain and thoracic ganglion of the giant freshwater prawn, Macrobrachium rosenbergii *de Man, were examined for the presence of specific acetylcholinesterase (AChE) by a histochemical technique. Neurons and axons containing AChE were detected in all the tissues studied. AChE-positive neurons were found in the medulla externa and medulla interna of the optic lobe; they were small monopolar neurons whose axons running proximally toward the optic peduncle. These axons, which also showed positive reactions for AChE, were detected in the medulla externa, medulla interna and optic peduncle, but not in the medulla terminalis. AChE-positive axons were seen as dark brown precipitate on axolemma along the width of the axons. In the optic peduncle, positive staining on axons was spared at intervals along the length of the axon fibers. A few small neurons and several nerve fibers in the brain were stained positively for AChE. The nerve fibers were of smaller width when compared to those of the optic peduncle. Regional distribution of AChE-positive fibers was also observed in the brain. Several nerve tracts in the thoracic ganglion were AChE-positive with dark brown precipitate along the axolemma, but without a characteristic sparing pattern as seen in the optic peduncle. The size of the axons were the same as that of the optic peduncle.*

INTRODUCTION

Crustacean neural tissues contain both neural and endocrine elements essential for several physiological regulations.[1] The optic lobe in the crustacean eyestalk is connected to the brain and contains neurosecretory cells collectively known as the X-organ; this organ produces several protein hormones which are carried along the axons to be stored and released at the terminals, the sinus gland.[1] Very little is known about the neural circuitry in the optic lobe and the control of the X-organ by the brain and thoracic ganglion. The nervous system of crustaceans has been shown to contain catecholamines, gamma-aminobutyric acid (GABA), encephalin and substance-P, 5-hydroxytryptamine (5HT), octopamine, proctolin, acetylcholine (ACh) and glutamate.[2-14] 5HT stimulates the release of molt-

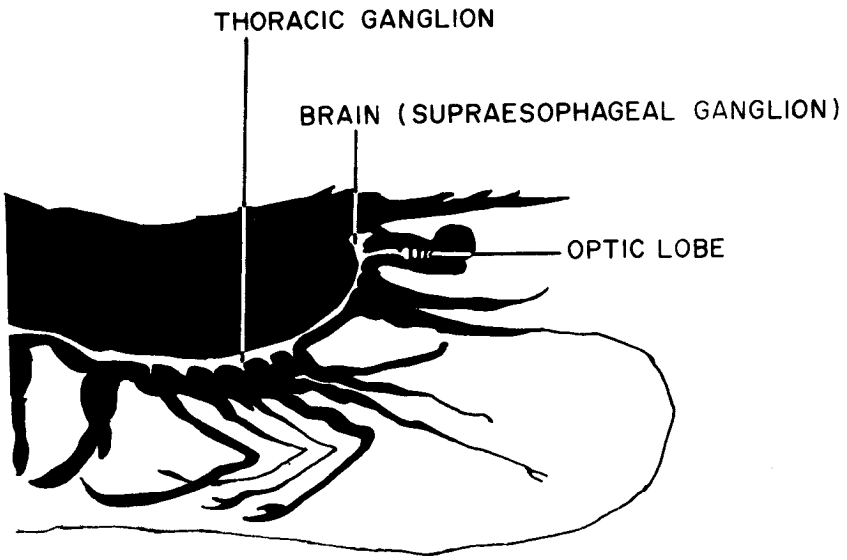

Fig. 1. A drawing showing positions of the optic lobe, brain and thoracic ganglion of the giant freshwater prawn, *Macrobrachium rosenbergii* de Man. These parts of the nervous system were isolated and examined for the presence of acetylcholinesterase in this study.

inhibiting hormone (MIH) from the sinus gland and is abundant in the organ of Bellonci of the optic lobe.[15, 16] ACh and choline acetyltransferase (CAT) were detected biochemically and histochemically in the optic lobe of the crayfish.[13] Linkage between cholinergic stimulation and endocrine regulation in the optic lobe of the crayfish was also observed.[17] These data suggest that ACh is one of the basic neurotransmitters in the nervous system of crustaceans. The report herein also describes the presence of specific acetylcholinesterase (AChE) in the nervous system of the giant freshwater prawn, *Macrobrachium rosenbergii* de Man.

MATERIALS AND METHODS

The optic lobe, the brain and the thoracic ganglion of the adult *M. rosenbergii* (Fig. 1) were isolated and processed for histochemical localization of AChE activity according to the method described by Koelle.[18] Briefly, the specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature for 15 min. They were washed thrice with the same buffer and then sectioned at 6-8 μm thickness in a freezing microtome. The sections were collected on gelatin-coated slides and immersed in the substrate base solution consisting of $CuSO_4.5H_2O$ 0.3 g, glycine 0.375 g, $MgCl_2.6H_2O$ 1 g, maleic acid 1.75 g, 1 N NaOH 30 ml and 40% Na_2SO_4 170 ml (pH 6) for 5 min. Pseudocholinesterase activity was inhibited by treating the sections with 10^{-5} M tetraisopropylpyrophosphoramide (iso-OMPA) in 40% Na_2SO_4 solution for 30 min. The sections were then incubated in acetylthiocholine iodide (ASChI) solution (ASChI 20 mg in 10 ml substrate base) for 2 hours, rinsed thrice in 40% Na_2SO_4 solution, immersed in 1% $(NH_4)_2S$ for 2 min and rinsed twice in distilled water. Finally, the sections were dehydrated in graded series of ethanol, cleared in xylene and mounted. Control sections were treated identically except that ASChI was omitted from the solution. The sections were photographed under an Olympus BH-2 microscope.

RESULTS

AChE-positive neurons were observed in the region of medulla externa and medulla interna of the optic lobe (Fig. 2), but not in the lamina ganglionaris and medulla terminalis. They were small monopolar neurons whose long axons were also AChE-positive. The axons were directed proximally and could be observed within the neuropile of the medulla interna, but not within the medulla terminalis. The AChE-positive axons were clearly observed in the optic peduncle (Fig. 3). When examined closely, These AChE-positive axons appeared as bands of dark brown precipitate outlining the width of the axons (1.5 μm), indicating that AChE was located on the axolemma. They appeared as segments of varying length due to different planes of sections. At intervals, the AChE-positive axons were deprived of the dark brown precipitate.

In the brain, AChE-positive axons were observed as neural tracts passing through the structure (Fig. 4). These axons were of smaller size than those observed in the optic lobe (0.5-1.0 μm). Certain parts of the brain contained these fibers but some did not, indicating

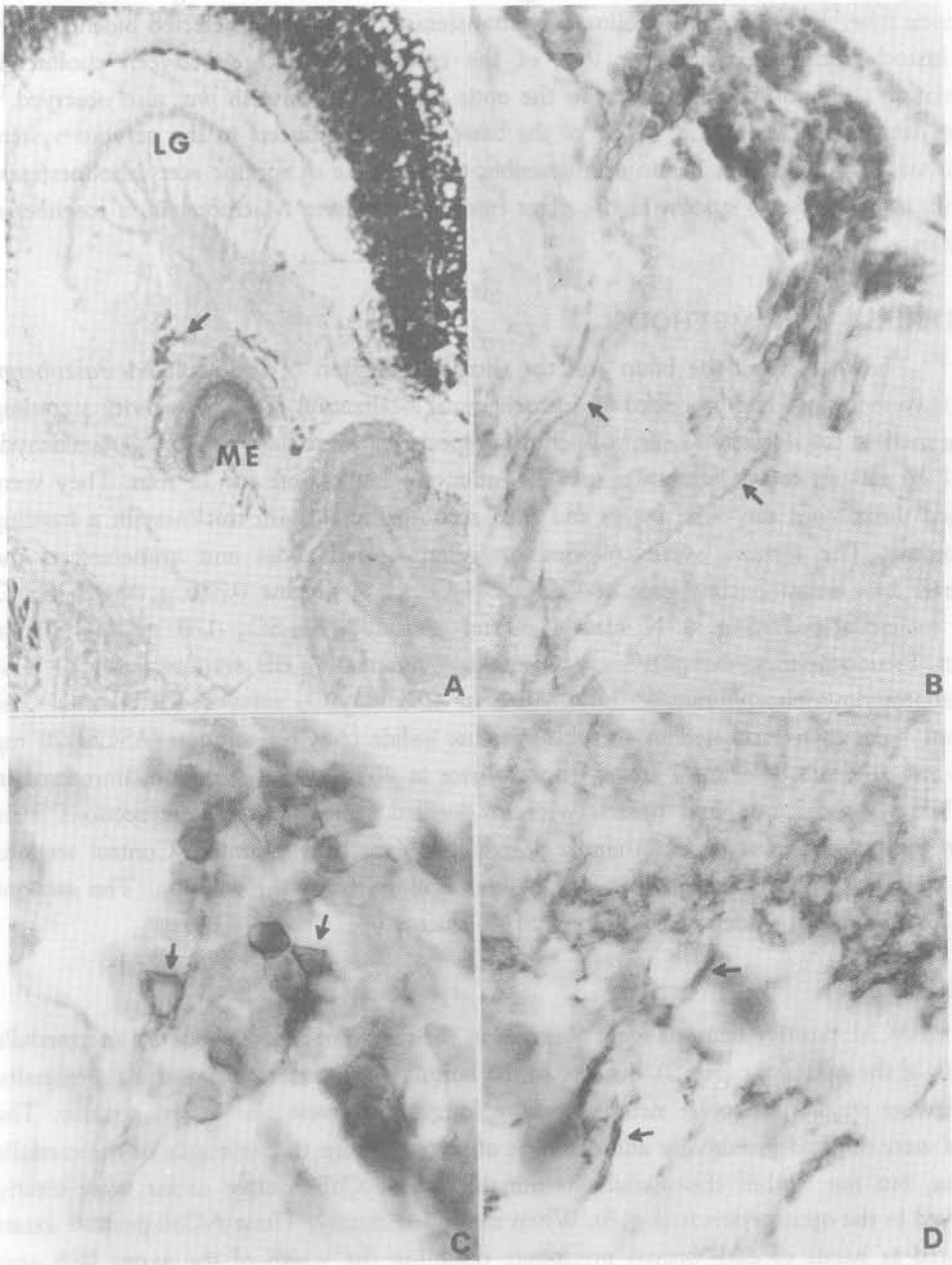

Fig. 2. AChE-positive neurons in the optic lobe. These neurons are localized in the medulla externa region (A, arrow) and send axons toward proximal parts of the optic lobe (B, arrows). AChE precipitate forms a dark brown border of these small angular neurons (C, arrows). AChE-positive fibers pass neuropile of the medulla externa and medulla interna of the optic lobe (D, arrows). LG, lamina ganglionaris; ME, medulla externa. Magnification: A, 40x; B, 400x; C, 1,000x; D, 1,000x.

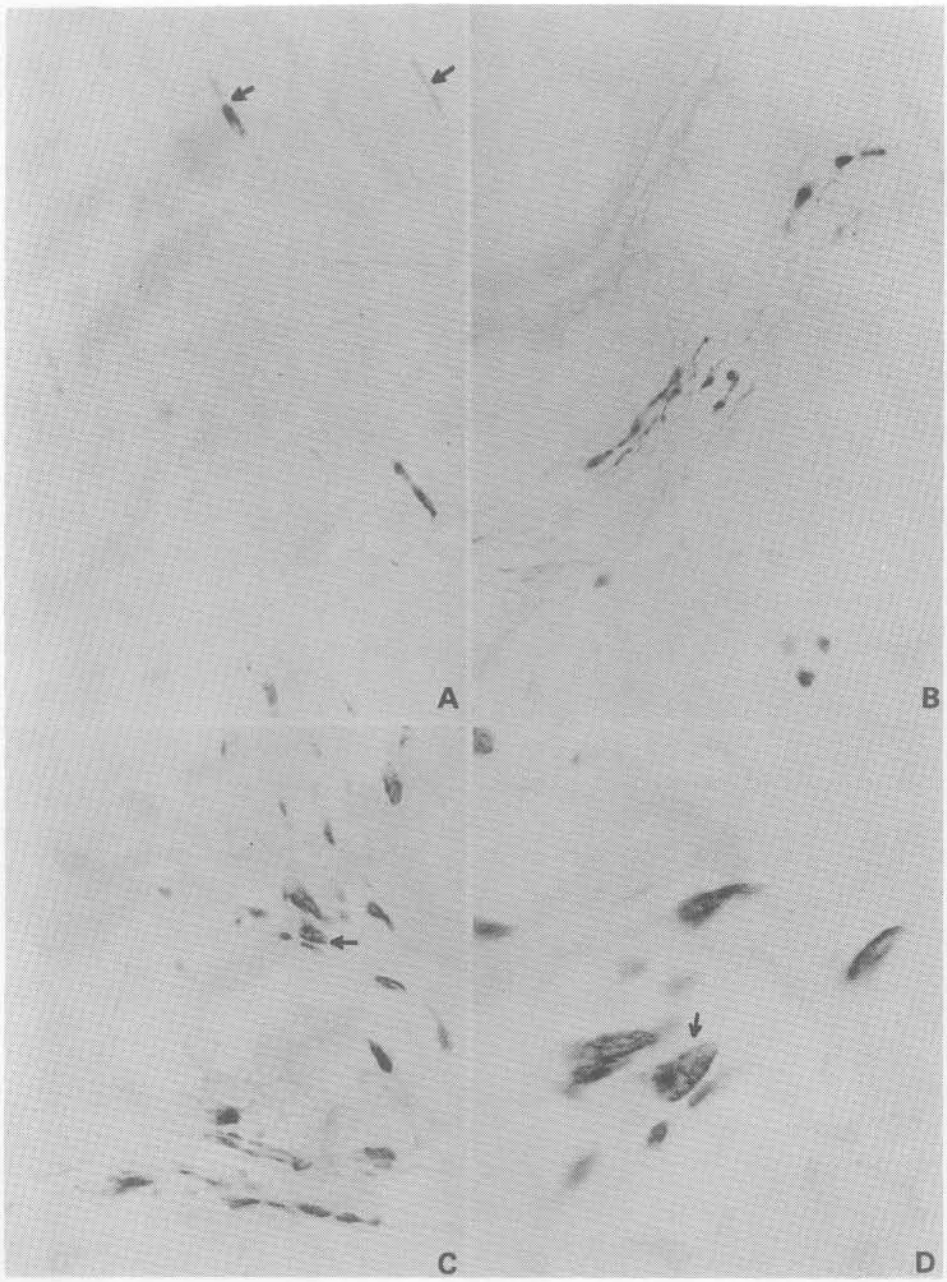

Fig. 3. AChE-positive fibers in the optic peduncle and medulla interna. At intervals, the fibers are spared of AChE precipitate (A, arrows). Certain nerve fibers (B) and small cells in medulla interna (C & D, arrows) and stained positively for AChE. Magnification: A, 400x; B, 400x; C, 400x; D, 1,000x.

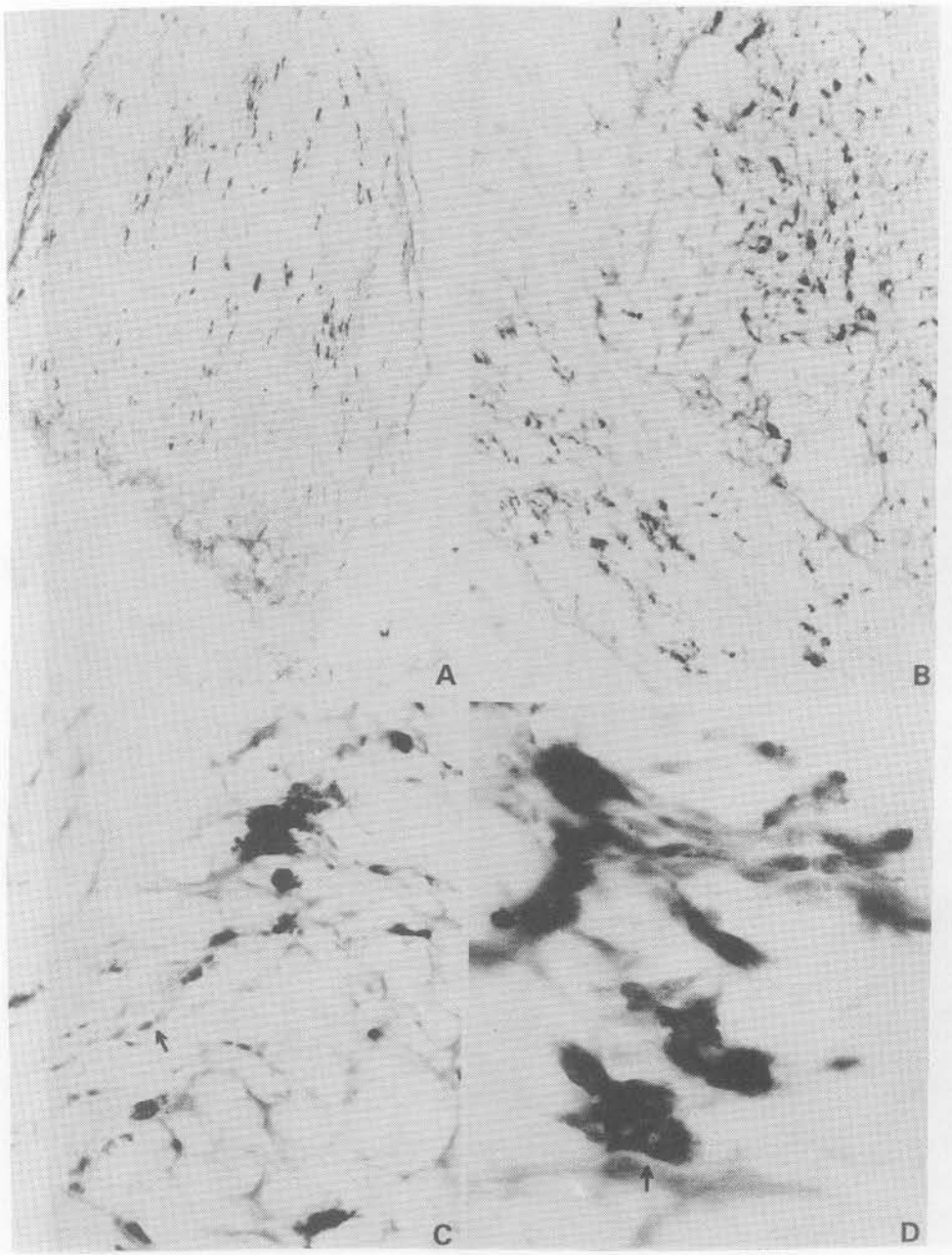

Fig. 4. AChE-positive fibers in the brain. Some positive fibers contain the interval sparing (C, arrow), the same feature as those observed in the optic peduncle. Certain small cells in the brain are also AChE-positive (D, arrow). Magnification: A, 100x; B, 200x; C, 400x; D, 1,000x.

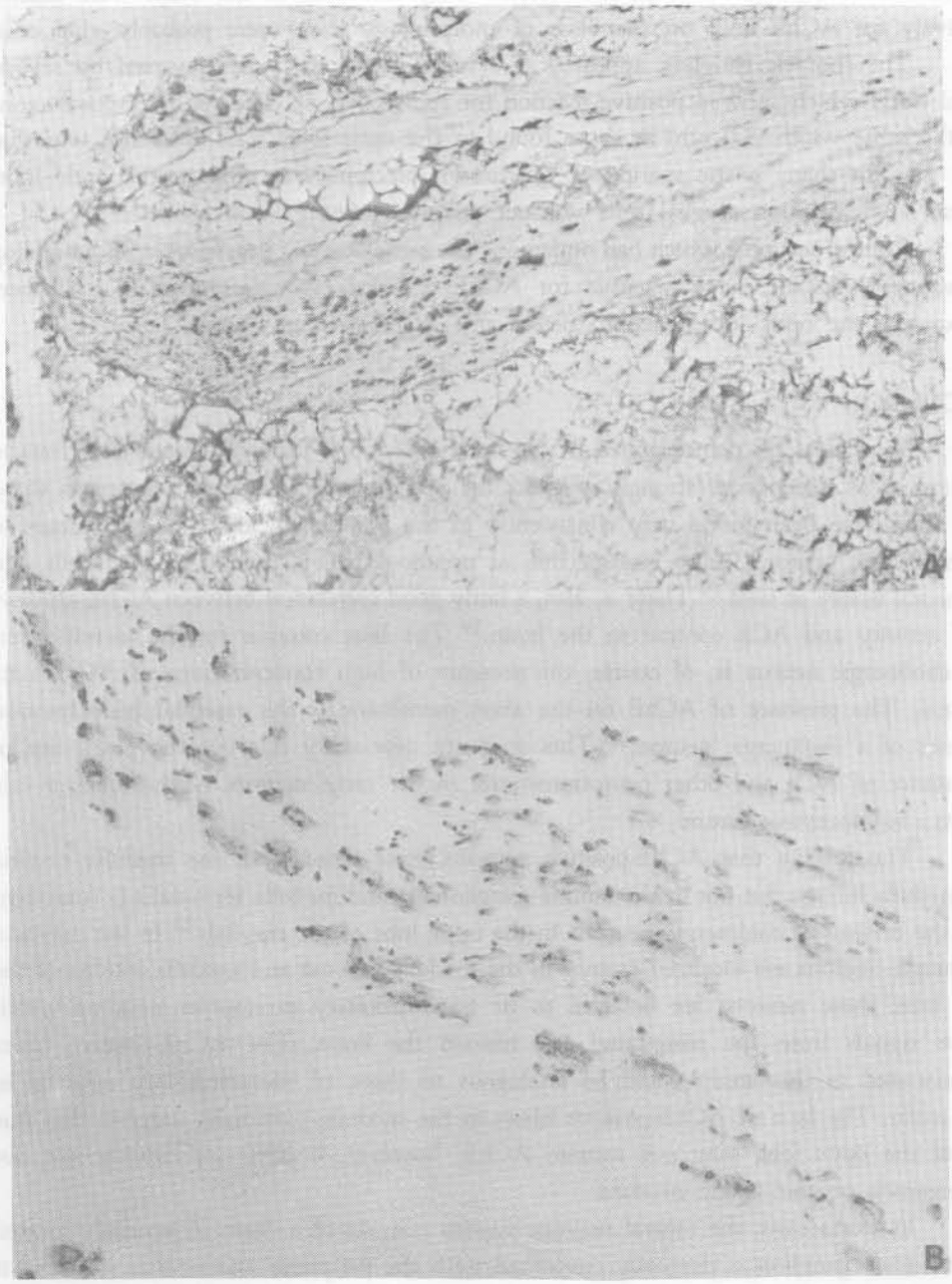

Fig. 5. AChE-positive fibers in the thoracic ganglion. Magnification: A, 40x; B, 200x.

regional distribution of AChE-positive axons. Very few cells in the brain were stained positively for AChE and, on the basis of morphology they were probably glial cells.

The thoracic ganglion appeared as a mass of neural tissue traversed by several neural tracts which showed positive reaction for AChE (Fig. 5). The AChE-positive axons had the same width (1.5 μm) as those found in the optic lobe. The difference was that there was no characteristic sparing of the brown precipitate as seen in the optic lobe. Several small neurons or glial cells without positive staining were observed.

Control sections, which had undergone the same staining procedure without adding the substrate ASChI, were negative for AChE. Careful examination of muscle fibers surrounding the optic lobe failed to detect any AChE-positive fibers.

DISCUSSION

Specific AChE demonstrated in the optic lobe, optic peduncle, brain and thoracic ganglion of *M. rosenbergii* strongly suggests the presence of cholinergic neurons in these sites. AChE has been found very consistently in the cell bodies, axons and terminals of all cholinergic neurons, while nonspecific- or pseudo-AChE is present in glial cells and non-neural tissues as well.[19] There is, also, a fairly good correlation between AChE activity, CAT activity and ACh content in the brain.[19] The best criterion for the identification of a cholinergic neuron is, of course, the presence of high concentrations of ACh in its terminal. The presence of AChE on the axon membrane is the essential histochemical evidence of a cholinergic neuron.[20] This does not necessarily rule out the possibility of coexistence of ACh and other neurotransmitter in the same neuron, neither does it rule out its cholinoceptive nature.

The finding that AChE-positive neurons were detected in the medulla externa and medulla interna but not in the lamina ganglionaris and medulla terminalis is consistent with the finding of cholinergic neurons in the optic lobe of the crayfish.[13] In the crayfish, cholinergic neurons are localized mainly in the medulla externa and medulla interna of the optic lobe; these neurons are believed to be transmedullary monopolar neurons which receive signals from the compound eye toward the brain. The AChE-positive fibers demonstrated in this study could be analogous to those of transmedullary neurons in their study. The lack of AChE-positive fibers in the medulla terminalis suggests that this part of the optic lobe may not contain AChE; however, it does not entirely rule out the presence of cholinergic neurons.

In crustaceans, the central nervous system consists of a chain of ventrally located ganglia. Each ganglion is primarily concerned with the peripheral tissues that surround it and communicates with these tissues through nerve roots containing efferent and afferent axons.[10] Neurons of each ganglion contain either neurotransmitters or neurohormones for use at peripheral targets: for instance 5HT and octopamine are localized in the thoracic ganglia,[10] 5HT is localized in the pericardial nerve plexuses.[8] These ganglia are interconnected by fibers which probably belong to transmedullary neurons, the neurons whose functions are analogous to small interneurons of vertebrates. Speculatively, ACh may be a primary

neurotransmitter of these interneurons since it has been found in several nerve tracts in the areas studied.

The significance of the sparing of AChE precipitate in the axolemma of the optic lobe fibers is not known. Its consistency suggests that this precipitative pattern is real, not artifactual. The sparing pattern gives an impression of axons with nodes of Ranvier at intervals. If this is the case, the AChE-positive areas would represent the myelin portion surrounding the axons. This is partially supported by the finding that some glial cells, in which myelin is produced, were stained positively for AChE. However, this hypothesis can only be solved by ultrastructural study of the nerve fibers.

AChE has not been found in muscle fibers, suggesting that muscle contraction is not controlled by cholinergic fibers. Unlike mammals, crustacean muscle activity is regulated by 5HT, octopamine, proctolin and dopamine.[10] 5HT, octopamine and proctolin induce contractions while dopamine relaxes the muscles.[21].

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บทคัดย่อ

จากการศึกษาหาตำแหน่งของเอ็นไซม์ acetylcholinesterase (AchE) ใน optic lobe, optic peduncle, brain และ thoracic ganglion ของกุ้งก้ามกราม ผลปรากฏว่าพบปฏิกิริยาของเอ็นไซม์ AchE ใน neurons และ axons ของ tissues ดังกล่าวข้างต้น AchE-positive neurons พบใน medulla externa และ medulla interna ของ optic lobe เป็น monopolar neurons ตัวเล็ก ๆ ซึ่งมี axon วิ่งไปสู่ optic peduncle Axon ที่แสดงปฏิกิริยาต่อ AchE เหล่านี้ ไม่พบใน medulla terminalis AchE-positive axon มีลักษณะเป็นสีน้ำตาลเข้มสะสมอยู่ที่ axolemma ตามความกว้างของ axons ใน optic peduncle ใน axon ที่ติดสีน้ำตาลมีจุดที่ไม่ติดสีเป็นช่วง ๆ ในสมอง พบ neurons ตัวเล็ก ๆ บ้าง และพบ nerve fiber เป็นจำนวนมากที่ติดสีน้ำตาล Nerve fiber ในสมองเมื่อเปรียบเทียบกับใน optic peduncle แล้ว มีขนาดเล็กกว่า ใน thoracic ganglion AchE-positive fiber ติดสีน้ำตาลเข้มตาม axolemma แต่ต่างจากลักษณะที่พบใน optic peduncle คือ ไม่มีจุดที่ไม่ติดสีเหมือนเช่นที่พบใน optic peduncle ขนาดของ axon เท่ากับที่พบใน optic peduncle