CHAPTER IX

CHOLINESTERASES IN THE CAUDAL NEUROSECRETORY SYSTEMS OF MIGRATING HILSA ILISHA (HAM.) AND IN NON-MIGRATORY HILSA TOLI (CUV. & VAR.)

Uemura, Kobayashi and Ishii(1963) reported acetylcholine or accetylcholine-like activities in the caudal neurosecretory storage organs of fresh water and marine fishes, and in bovine parsnervosa; suggesting role of Acetylcholine in secretion. The presence of cholinergic substance in the caudal neurosecretory system may be due to the presence of synaptic vesicles demonstrated by electron microscope studies of fish caudal neurosecretory organ(CNS),(Kobayashi, Uemura,Oota, and Ishii, 1963), as these vesicles were similar in appearance to acetylcholine bearing vesicles of the brain, which are supposed to be the carriers of acetylcholine (De Robertis, Salgonicoff, Zieher and Rodriguez De Lores Arnaiz, 1963).

Pearse (1958) observed an increase in acetylcholinesterase (AchE) activity in the perikarya of supraoptic neurosecretory cells of rat kept on salt diet, the effect of salt diet on neurosecretory cells (NS cells) is well known. Simultaneous decrease in AchE activity in magnocellular neurosecretory cells along with the decrease in release of antidiuretic hormone was observed by Kivalo,Rinne

and Makela (1958) due to effect of Chloropromazine. The release of neurohypophyseal hormones from the neurosecretory cells as the result of intravenous injection of acetylcholine in dogs was reported by Pickford(1939,1947) and Abrahams and Pickford (1954). All these observations may point out a direct or indirect relationship of acetylcholinesterase (AchE) activity with the secretory activity of neurosecretory cells. The cholinergic substance may be specifically concerned with the release of neurohormone as suggested by several workers (Koelle,1961; Gerschenfeld Tramezzani and De Robertis, 1960). Arvy (1962),Kobayashi and Farner (1964) also supported the above mentioned hypothesis by demonstrating cholinesterase in neurosecretory cells of mammals and bird, respectively.

It is believed that the CNS of fishes play important role in osmoregulation (Chapter VIII). It would be significant and interesting, if the cholinesterases activity is studied in the CNS of an anadromous migratory fish and a comparision is made with a closely associated nonmigratory fish and of nonmigratory fish drifted into estury by the force of highest high tide. This may throw light on functional significance of cholinesterases in relation with the different states of activities of neurosecretory cells believed to be concerned with osmoregulation.

MATERIALS AND METHODS

Fishes were captured and sacrificed within two minutes after capture from the nets and immediately the spinal cord in the last eight vertebrate alongwith urophysis was kept in chilled formal saline (Gurr, 1956) for 10 to 12 hours. The fixation time was uniformly maintained throughout this study and this investigation. The material, then, was washed with distilled water thoroughly, till the formalin was completely removed. Gelatin blocks were prepared and after storing the blocks in 1% cold formalin for 48 hours in freeze, 15 μ sections were cut with freezing microtome. Sections were also washed with distilled water for 20 to 25 minutes to remove formalin. They were then incubated. Method as modified by Coupland and Holmes (1957) was followed for the demonstration of cholinesterases. The sections of the spinal cord alongwith the urophysis were incubated separately for the demonstration of two enzymes; AchE and butyrylcholinesterase(BchE) in the neurosecretory cells, urophysis and blood vessels. Acetylthiocholineiodide and butyrylthiocholineiodide (Sigma Chemical Co.,U.S.A.) were used as the respective substrates for AchE and BchE. The pH of the medium was maintained between 5.6 and 6.0 as optimal precipitation of the crystalline deposits of copper thiocholine sulphate is said to occur at that pH (Malmgren and Sylven,1955).

The incubation was done at 37°C and incubation time was varied according to the concentration of the enzymes present in the tissue and according to the stages of secretory activity of the neurosecretory cells.

The control sections were kept at 37°C for 2 to 3 hours, with 3x10 M solution of eserine sulphate which acts as an inhibitor to both the enzymes. The time of fixation, incubation, pH of media etc. precautions are taken into consideration asper recently published literature (Naik,1963).

RESULTS

The neurosecretory cells of the CNS of Hilsa ilisha captured from sea, (stage II and III of maturity) were in active state of secretion and NSM is continously transported into the urophysis by axon fibres (Chapter VIII) as stained by Acid-Violet staining technique - A.V. staining (Takasugi and Bern, 1962). The neurosecretory cells of the Hilsa ilisha (stage II and III of maturity) captured from sea showed +++ activity in the perikarya and ++ activity in some axon fibres and ++++ in most of the axon fibres. BchE activity can be represented by ++ in perikarya of neurosecretory cells, ± activity in axon fibres. The incubation time was according to the Table I.

The neurosecretory cells of the spinal cord of Hilsa ilisha captured from river at the time of migration (stage V and VI of maturity) exhibited ++++ activity of AchE in perikarya of NS cells and in axon fibres also (Fig.1). ++++ activity of AchE was also shown by neurosecretory cells situated in the urophysis. These neurosecretory cells of spinal cord and urophysis, in paraffin sections, when stained with A.V. staining showed exhaust phase and discharing phase, the prominent transport of NSM from these cells to the urophysis suggest heavy synthesis and transport of NSM to the urophysis in blood vessels (Chapter VIII). All these activities of both enzymes may be in relation with the neurosecretory activities of the neurosecretory cells.

The caudal neurosecretory cells of spent H. ilisha showed complete exhaust phase, no neurosecretory granules were visible in them, many of them are shrunk, while few of them had started resynthesis of NSM as evident by presence of NS granules in the nucleus itself. Urophysis is devoid of NSM, as all NSM is discharged in the blood stream. The NS cells show +++ activity, in axons and +++ activity in perikarya, for AchE activity. It was remarkable to note that +++ activity is seen in axon fibres upto very long distance for AchE. BchE showed similar intensity of the histochemical reaction in perikarya of NS cells, +++ in axon fibres, and + in axon fibres

region situated far away from NS cells proper. ++ activity was shown in the blood vessels of the bulb (Fig. 2), and the spinal cord also.

In immature H. toli captured from the sea, (stage II and III of maturity) the AchE activity can be represented by ++++ in perikarya of NS cells, ++++ in axon fibres and ++ in perikarya some NS cells. BchE activity is represented by ++++ activity in perikarya of NS cells, by ++++ in axon fibres, ++ in blood vessels. When paraffin sections, stained with A.V. staining, the NS cells of spinal cord many of them were in state of active secretion, many of them are discharing NSM into the urophysis. This is revealed by A.V. staining.

++++ activity, for AchE is represented in perikarya of NS cells and +++ activity in axons of NS cells of mature Hilsa toli (stage V and VI of maturity). BchE could be symbolised by +++ activity in axons of neurosecretory cells and by ++ activity in blood vessels (as noticed in Figs.3,4). In paraffin sections, the NS cells showed discharge phase and some of them were observed in active state of secretions,when stained with A.V. staining. NSM could be visualised as transported to the urophysis by axon fibres. NSM was also seen discharged into the blood stream.

In spent Hilsa toli captured from the sea (Stage VII of maturity), the NS cells were found to be in exhaust phase

and discharged NSM transported into the bulb. Many blood vessels had appeared. The urophysis was devoid of NSM and in few sections, little NSM could be seen at the peripheri of the urophysis. AchE activity was represented by ++++ in perikarya of NS cells of spinal cord and ++ activity was observed in axons of the NS cells. BchE activity was symbolised by ++ in perikarya of NS cells and by ++ in axons of the NS cells. +++ activity was shown by walls of the all blood vessels (As in Fig.2).

It was interesting to note ++++ AchE activity in the perikarya of NS cells of spinal cord and ++++ activity in axon fibres extending upto very long distance and +++ activity in shrunk NS cells of spinal cord of mature H. toli (stage V and VI of maturity) drifted into the river Narbada on the day of highest high tide of the year, which was captured from Bhadbhoot estuarine zone (Refer map 2 of Chapter II). BchE activity was represented by ± in perikarya of NS cells of spinal cord and in axon fibres also. +++ activity was demonstrated in all the walls of the blood vessels. +++ activity of acetylcholinesterase demonstrated in the walls of blood vessels, which were extending upto very long distance. In paraffin sections, the NS cells of drifted Hilsa toli, showed tremendous discharge of NSM and noticeable transport of NSM was observed in axon fibres. Almost all NS cells were shrunk

and most of them were in 'exhaust phase' also. Few of them were degenerated leaving empty spaces behind, urophysis was found devoid of NSM and in few sections, NSM was seen on the peripheri of the urophysis. All these changes may due to change in salinity and in different ion concentrations.

The results obtained can be tabulated as follows to obtain an idea about the activity of cholinesterases in relation to the activities of NS cells of the CNS of the migratory Hilsa ilisha and the non-migratory Hilsa toli.

DISCUSSION

The results tabulated above shows the presence of the cholinesterases in the perikarya of the neurosecretory cells, in the axon fibres of the neurosecretory cells, and in the walls of the blood vessels. Uemura, Kobayashi and Ishii (1963) on basis of observations made by electron-microscope on the CNS of carp, suggested that the high concentration of AchE may be related with the release of neurohormones produced by the neurosecretory cells, in addition to the above mentioned hypothesis, the same authors have also put forwarded the probable region of activity of AchE in the membrane of NS granules, in the cell membrane of axon-endings or in the walls of capillaries. The results

obtained by us support the above mentioned views. Several workers have also suggested the role of cholinesterases in secretory activities of the neurosecretory cells (Kivalo, Rinne and Makela, 1958; Koelle, 1961; Gerschfeld, Tramezzani and De Robertis, 1960; Arvy, 1962; Kobayashi and Farner,1964). From the results obtained from these investigations, it may be tried to correlate the activities of the neuroscretory cells, i.e. in active state of secretion and when NSM is synthesized by them, is being transported into the urophysis. The strong activity of AchE may be related to the active synthesis and transport of neurohormones synthesized by NS cells of the CNS of both the fishes.

When migratory Hilsa ilisha ascends into the river Narbada from the sea for spawning, there is a significant downfall in Na, K, Ca and chlorides concentration as evident by water analysis (Refer column two of Table I) and if the CNS may be involved in osmoregulation (Chapter III). The NS cells of the spinal cord might release NSM from the urophysis and from perikarya of the NS cells also. This correlation of the activities of release of neurohormones from NS cells of the spinal cord of migrating Hilsa ilisha(Ham.) may be due to change in ions dissolved in the river waters and strong activities localized in the NS cells of spinal cord supports the views held by several workers that AchE is directly or

indirectly concern with the release of neurohormones from the neurosecretory cells.

When Hilsa ilisha returns to sea after laying eggs in fresh water zone of river Narbada, the NS cells of the spinal cord showed 'exhaust phase' and some showed resynthesis of NSM in them, very little NSM in urophysis, all these results obtained from A.V. staining (Chapter VIII) procedure showed that neurohormones were released fully into the blood stream and synthesis and manufacture was resumed by NS cells of the spinal cord in spent Hilsa ilisha returning to sea. The presence of shrunk and degenerated cells and empty spaces left by degenerated cells in the spinal cord supports the above view. Appearance of many blood vessels might be for blood supply for resynthesis of NSM. AchE activity is strongly localized in almost all NS cells (++++ activity in 10 hours time in perikarya) moderate activity in an axon fibres (+++ in 18 hours incubation time) medium activity was observed in the walls of blood vessels (++ activity in 20 hours of incubation). BchE activity was found to be moderate in perikarya of NS cells (+++ activity in 18 hours) and in blood vessels also (+++ activity in 19 hours of time of incubation). The presence of BchE as well as AchE activity in blood vessels suggest that there may be heavy blood supply in the CNS this is also

supported by acid violet staining (Chapter VIII).

The spent Hilsa toli captured from sea gave interesting results, but the results were similar to spent Hilsa ilisha captured from the river Narbada. The NS cells were in 'exhaust phase', except few in regaining phase of secretion, NSM was transported into the urophysis, which was exhausted. AchE activity was found strong in perikarya of NS cells (++++ activity in perikarya in 11 hours of incubation time) and medium in axon fibres (20 hours time of incubation); this may be correlated with the activities of NS cells. BchE activity was found to be very weak in perikarya and axon fibres of NS cells (+ activity after 20 hours incubation time) but moderate activity of BchE was demonstrated in the blood vessels (+++ activity after 18 hours incubation) later may be due to blood supply for resynthesis of NSM, as NSM was found to be very little in urophysis of spent Hilsa toli (Chapter VIII).

When histological and cytological pictures of CNS of mature Hilsa toli captured from river on the day of highest high tide day were compared with those CNS of Hilsa toli of same stage of the maturity captured from the sea, very interesting results were obtained, which may throw light on the probable osmoregulatory role of the CNS. The NS cells of former, were found to be in 'exhaust phase',many were

found shrunk and destroyed leaving empty spaces behind and muscle fibres alongwith axon fibres gave wavy appearance forming many lacunae. Very little quantity of NSM was observed in the urophysis, but transport of NSM from the spinal cord was noticeable. The same species, Hilsa toli, of same stage of maturity, captured from the sea showed very less degenerative changes.

As stated in Chapter VIII, these sharp changes may be due to change or downfall in Na, K, Ca, and chlorides contents of river waters, which is clearly mentioned in the tables I and II. Ache activity was very strong in perikarya of NS cells, in shrunk NS cells of the spinal cord, in axon fibres and in walls of blood vessels also (++++ in perikarya, ++++ in shrunk NS cells, ++++ in axon fibres and ++++ in walls of blood vessels in 10 hours of incubation). The strong activities of AchE may be confined to the strong and heavy discharge of NSM from the NS cells, which may be playing important role in osmoregulation. On the other hand BchE activity was also moderate in perikarya of NS cells, and in blood vessels (+++ in perikarya of NS cells, +++ in the walls of blood vessels of Hilsa toli captured from river). The NS cells of mature Hilsa toli (stage V - VI of maturity) captured from the sea, showed 'discharing phase' and NSM was transported into the urophysis, few NS cells were in 'exhaust phase' too. AchE

activity was strong in perikarya of NS cells (++++ activity in 10 hours)and was moderate in axon fibres (+++ activity in 18 hours). BchE activity was found to be moderate in perikarya of NS cells and medium in blood vessels (+++ activity in 19 hours in perikarya and ++ activity in 20 hours in blood vessels).

From all above mentioned observations, it may be stated that AchE alongwith BchE may play important, direct or indirect role in release and synthesis of neurohormones. It might have important role in nerve tissue metabolism as cholinesterases were strongly localised in NS cells of 'exhaust phase' also. This observation supports the observation of Hokin and Hokin (1956).

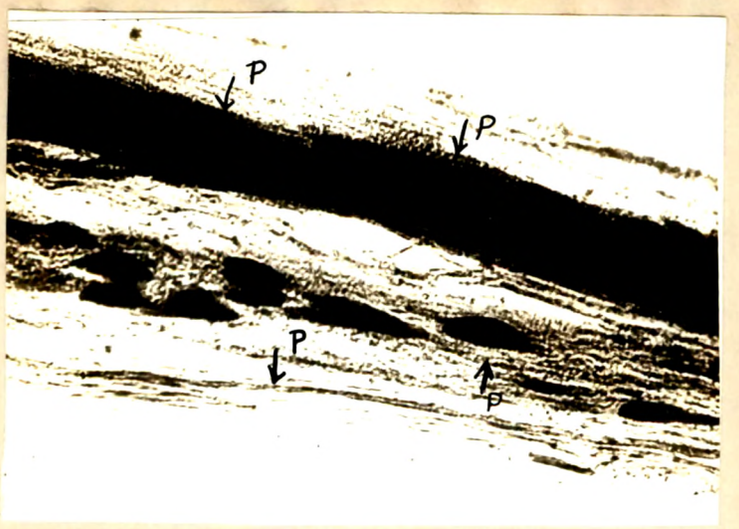

Fig. 1

AChE of the Caudal Neurosecretory cells and of the (P) pathways of Caudal Neurosecretory system of mature migrating H. ilisha.

Substrate, AThCh, x160

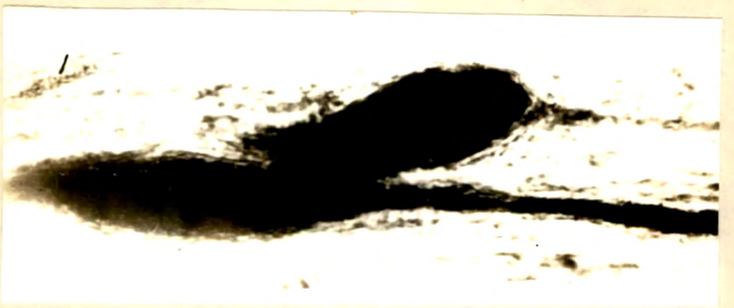

Fig. 2

BChE of the Caudal neurosecretory cells of the Caudal Neurosecretory System of mature, migrating H. ilisha.

Substrate, BuThCh, x160.

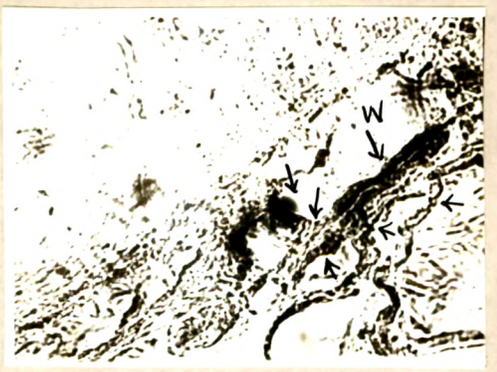

Fig. 3

AChE of the wall of the blood vessels of the caudal neurosecretory system of mature, H. ilisha.
Substrate, AThCh, x400

W - Wall of the blood vessel.
Arrow indicates termination of the axon on the wall of the blood vessel.

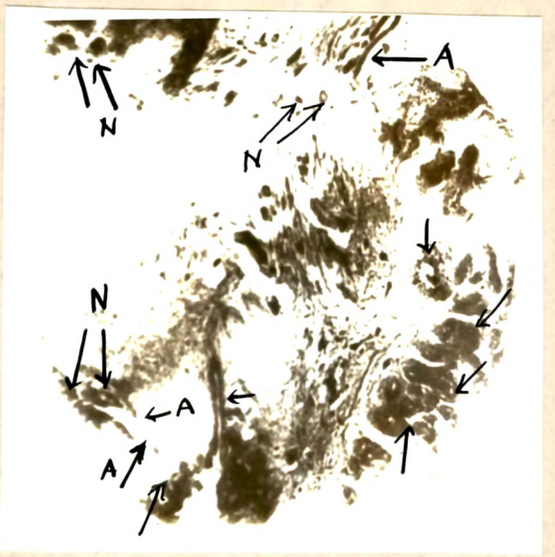

Fig. 4

AChE of the axons of the NS cells and of the blood vessels of the urophysis of the mature, H. ilisha.
Substrate, AThCh, x63.

N - NS cells
A - Axons
(Arrow indicates wall of the blood vessel)