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Histoenzymological Distribution of Acetylcholinesterase in the Rostral Mesencephalic Torus Semicircularis and Tegmental Nuclei of an Indian air Breathing Teleost *Heteropneustes fossilis*

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ABSTRACT

Mesencephalon or mid brain of the fishes consists of dorsal roof or optic tectum and ventral roof or tegmentum. Optic tectum (OT) in the presently studied specimen is a bilobed structure which is composed of six stratified zones of differently shaped and differently sized neurons. Tegmental area is the major component of the mid brain of fishes which is responsible for diverse functions and it comprises many cholinergic and cholinoceptive nuclear groups particularly the rostral tegmental area in which many caudal thalamic and hypothalamic nuclei diffuse. Enzyme acetylcholinesterase (AChE) which hydrolyses the neurotransmitter acetylcholine in to choline and acetate at the synaptic cleft is a prime marker of cholinergic-cholinoceptive neurons in the vertebrate central nervous system. In the present study a histochemical technique was carried out to locate AChE in the tegmental area described by Hedreen J.C. *et.al* (1985).

In the tegmentum of *Heteropneustes* the density of acetylcholinesterase stained cells was very high. In the dorsal and rostral tegmental nuclei some AChE positive cells were observed which were abundant in latter. The nucleus anterioris tuberis and nucleus lataralis tuberis which are the hypothalamic nuclei diffused in mesencephalic area displayed very intense AChE positive cells while central pretectal nuclei exhibited medium to weak stained cells. Dorsal posterior nucleus and central posterior nucleus of thalamus also showed intensely AChE stained cells. AChE positive cells were observed in the whole rostro-caudal extension of the tuberal area and lateral recess nuclei. The present results corroborate the previously reported connection between the rostral tegmental nucleus and the nucleus of thalamus and pretectal area and to the hypothalamus on the basis of degree of AChE intensity. Present findings have also been discussed in the light of previous findings on other vertebrate groups.

Keywords: Tegmentum, Torus Semicircularis, Cholinergic, Cholinoceptive, Mesencephalon, Thalamic, Tuberal area

INTRODUCTION:

Teleosts which represent the most prominent and diversified group among actinopterygians exhibit a complex nervous system in terms of cyto-architecture, hodology and number of neurons. The distribution of enzyme acetylcholinesterase in the forebrain and hind brain of *Heteropneustes fossilis* has been studied in detail by the present author (Tripathi and Rahman, 2014, 2016; Rahman et.al.2013).The present work is the extension of the previous study in which the mid brain remained unstudied.

Mesencephalon or mid brain of the presently studied fish consists of dorsal roof or optic tectum and ventral roof or tegmentum. Tegmentum further consists of torus

semicircularis and tegmental nuclei. The anterior part of the cerebellum (metencephalon) extends between the cavityof optic lobes as corpus cerebella. Optic tectum which is comparable to the cerebral cortex of mammals and is a eye body coordinating centre of fishes has been described in earlier study (Tripathi et.al.2013). The cerebellum of the presently studied fish which is visible in figure-1, has also been described earlier by the present author (Rahman et.al.2013). Thus the present study is focused on the distribution pattern of AChE in the torus semicircularis and tegmental nuclei of the mesencephalon which still remains less explored.

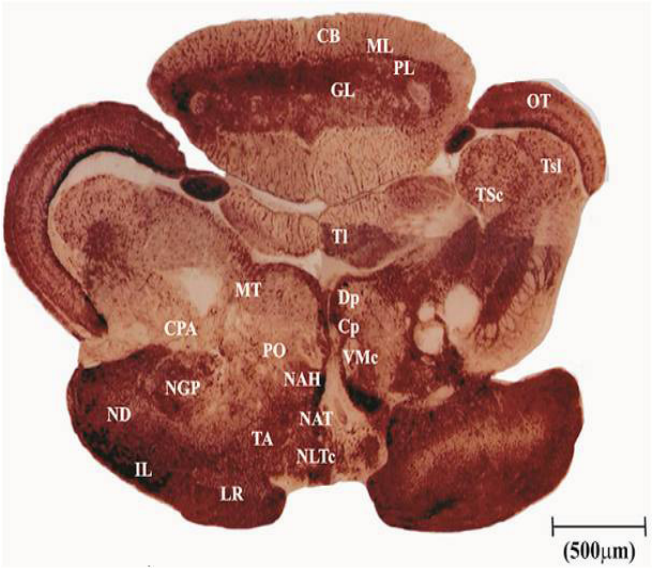

Fig. 1 : Photomicrograph of 30µm thick cryocut transverse section passing through rostral mesencephalon showing AChE activity in cerebellum, pretectal & tegmental nuclei. 4X

MATERIALS AND METHODS:

Eight adult *Heteropneustes fossilis* weighing 35-40 gram and length between 16-20 centimeter were used in the present study. The animals were acclimatized according to laboratory conditions before sacrificing them. Experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee (IAEC) of the Kulbhaskar Ashram P.G.College,Prayagraj. The fish were anesthetized with MS-222 (Sigma, St. Lovis, MO) and decapitated. Brains were fixed in the solution of 0.5% Paraformaldehyde and 1.5% Gluteraldehyde in 0.1 M phosphate buffer (pH 7.4) for 6 hours at 4^0C. The mid brain tissue was then given 2-3 changes in 15% sucrose solution in 0.1M phosphate buffer and stored in the same solution for 2-3 days. 30 micron thick frozen sections were cut by A O Histostat at -22^0C and stored serially in 0.1 M phosphate buffer. AChE histochemistry was carried out by using a modified histochemical technique(Hedreen et.al.1985). The dark brown coloured patches appeared in sections which designated AChE activity. Omission of the substrate acetyl-thiocholine iodide from the incubating mixture was carried out as control for the AChE histochemistry and no residual activity was observed in controlled experiment.

RESULT AND DISCUSSION:

As shown in figure-1, In *H. fossilis* the transverse section passing through the rostral mesencephalon exhibits Corpus cerebellum (CB), Optic tectum(OT) and tegmentum. The description about CB and OT is being omitted here since the author has already described it earlier(Tripathi et.al.1913;Rahman et.al.2013). Tegmentum consists of Torus semicircularis (TS) and tegmental nuclei. TS region can be distinguished in to central (TSc) and lateral (TSl) cell groups, located below optic tectum(Fig.1).. The central part of the torus semicircularis consists of medium sized round to oval, scattered soma with intense AChE activity. The lateral part (TSl) also comprises same type of cell groups with scattered cells and these cell groups show rostrocaudal extensions and are ventrally oriented towards tegmental nuclei. These cells demonstrated intense reaction. Torus longitudinalis showed few small sized AChE stained cells.

In the tegmentum, the density of AChE stained cells was very high and their neurons showed different sizes. In the dorsal and rostral tegmental nuclei, the AChE intensity was very high in large sized somata. These cells showed fusiform or round cell bodies. The intensity of AChE in the different nuclei has been shown in table-1. AChE positive cells were observed in the whole rostrocaudal extension of the Tegmental mesencephale(MT) and nucleus glomerulus posterioris(NGP). These cells showed large sized somata with very intense AChE activity. The mesencephalic centre of the nucleus arcuatus hypothalamicus(NAH), nucleus lateral tuberis(NLT) and nucleus anterioris tuberis(NAT) showed very intense reaction for AChE. In addition, hypothalamic inferior lobe(IL), lateral recess(LR), and tuberal area (TA) showed very high intensity for AChE. The nucleus of the dorsal posterior(DP) and central posterior(CP) showed intense AChE stained cells with large sized round somata. Nucleus of the central pretectal area (CPA) however, demonstrated moderate activity for AChE. The activity of other nuclear groups and mesencephalic areas has been shown in table-1 and Figure-1

Table 1 : Rostral Tegmentum area of *Heteropneustes* showing AChE activity

Sl. No.	Name of Nuclei Torus Semicircularis	Abbreviation	AChE activity
1.	Torus semicircularis centralis	Tsc	+ + +
2.	Torus semicircularis lateralis	Tsl	+ + +

3.	Torus longitudinales	TL	+ +
4.	Tegmentum mesencephale	MT	+ + +
5.	Dorsal posterior thalamic nucleus	DP	+ + +
6.	Central posterior thalamic nucleus	CP	+ + +
7.	Ventromedial thalamic nucleus	VMc	+++
8.	Paraventricular organ	PO	+ +
9.	Nucleus arcuatus hypothalami	NAH	++++
10.	Nucleus anterioris tuberis	NAT	++++
11.	Nucleus lateral tuberis	NLT	++++
12.	Tuberal area	TA	++++
13.	Central pretectal area	CPA	+ -
14.	Nucleus glomerulus posterioris	NGP	+++
15.	Diffused nucleus of IL	ND	+++
16.	Inferior hypothalamic lobe	IL	++++
17.	Lateral recess	LR	++++

Notation:

++++	=	Very Intense	+++	=	Intense
++	=	Moderate	+—	=	Mild
— —	=	Negative			

Among the torus semicircularis subdivisions, central and lateral subdivisions in *Heteropneustes* showed intense activity for AChE while torus longitudinales showed moderate activity while in *channa* this subzone exhibited total negativity. In zebra fish the ventrolateral nucleus of torus-semicircularis also demonstrated AChE positive cells(Clemente et.al.2004).

The torus semicircularis is a sensory integrative mesencephalic area which acts as a target for the ascending octavolateralis system(Wullimann et.al.1996). The ventrolateral nucleus of the torus semicircularis receives afferants from the medial and caudal octavolateral nucleus; both are abundant in AChE positive cells but the same nuclei are ChAT immunoreactive in zebra fish(Clemente et.al.2004).These nuclei are included in the mechano-receptive ascending pathway. Cholinergic axons from the isthmic nucleus project to the optic tectum throughout the torus semicircularis(Philip 1968).Some of these neurons could make synapses with AChE positive neurons of the torus semicircularis suggesting cholinoceptive nature of AChE containing cells in this mesencephalic area.

Most of the tegmental nuclei in the presently studied fish showed very high intensity for AChE except central pretectal area which showed moderate to mild reaction. These results are in agreement with zebra fish tegmentum where most of the nuclei are AChE positive (Clemente et.al.2004). It is interesting to mention that most of the thalamic and posterior hypothalamic nuclei residing at tegmental areas exhibited strong reaction for AChE. Thus these centres represent the interface between tegmentum and diencephalon and most of them are cholinergic in nature executing various functions in coordination. But in zebra fish ChAT (Choline-acetyl transferase) immunoreactive cells were observed only in rostral tegmental, oculomotor and trochlear neuclei(Clemente et.al.2004). In most of the fish analyzed hitherto including our present study, NAH, NAT, NLT, DP and CP showed AChE activity. In addition, many of those fishes also showed ChAT immunoreactivity (Ekstrom 1987; Brantley et.al.1988; Anadan et.al. 2000; Adrio et.al.2000).our results thus corroborate the fact that few cranial nerve motor nuclei and many posterior hypothalamic and thalamic nuclei terminating in rostral tegmental centres are cholinergic in nature throughout fish evolution.

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