Distribution pattern of acetylcholinesterase in the optic tectum of two Indian air breathing teleosts

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KEY WORDS

Acetylcholinesterase
Optic tectum
Cholinergic
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ABSTRACT

Background: A histoenzymological study has been carried out on the distribution of enzyme acetylcholinesterase in the optic tectum of two Indian air breathing teleosts by employing a histochemical technique to visualize acetylcholinesterase containing neurons described by Hedreen, JC (1985). Purpose: Data available on enzyme localization in the brain of fishes, particularly Indian teleosts is inadequate and scattered. Methods: AChE distribution in the optic tectum shows a prevalent pattern characterized by precise laminar distribution of enzyme which shows alternatively strong, weak or negative reaction in the different layers. Results: Layers with maximum enzyme activity most likely correspond to areas where cholinergic mechanism is prevailing whereas layers with mild activity may be considered to be non-cholinergic/cholinoceptive having some cholinergic innervations from other layers. Conclusion: The present investigation suggests some possible connections between enzyme localization and functional and anatomical organization of optic tectum.

Introduction

Acetylcholinesterase (AChE) is a lytic enzyme belonging to the family of type B carboxylesterase that hydrolyses the neurotransmitter acetylcholine (ACh) in to choline and acetate.1 This enzyme is in abundance in the synapse of cholinergic neurons, playing a key role in the cholinergic neurotransmission. Recently, additional non cholinergic functions of AChE have been elucidated.2–4 The two roles of AChE (Cholinergic and non-cholinergic) provide adequate ground to functionally correlate its distribution in the different brain centers.

The distribution of cholinesterases has been carried out in the brain of several mammalian,5–10 avian,11–15 and reptilian16–19 species. Data available on enzyme localization in the brain of fishes, particularly Indian teleosts is inadequate and scattered.

For this reason, a study was initiated on the enzymatic distribution pattern in some brain regions of two Indian air breathing teleosts (Channa punctatus and Heteropneustes fossilis). Our research starts with histochemical distribution of AChE in the optic tectum (OT) of above mentioned species based on recent cytoarchitectonic and hodological studies. The neuroanatomical nomenclature of optic tectum follows that of Meek and Schettart, 197823 as adopted by Castro et al, 2006.24

Methods

Ten adult males of Heteropneustes fossilis (Length 16 ± 18 cm, weight 35 ± 40 gm) and Channa punctatus (length 15 ± 17 cm, weight 45 ± 50 gm) were collected from the natural habitat of Ranchi district and acclimatized for laboratory. All the experiments were carried out according to ethical guidelines of Ranchi University, Ranchi.

Animals were anesthetized with 0.2% 2-phenox ethanol. Fishes were perfused transcordially with 500 ml solution of 0.5% paraformaldeyde and 1.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.4). Brain was dissected out and post fixed in the same solution for six hours. Brain was then given 2,3 changes in 15% sucrose solution in 0.1 M phosphate buffer and stored in the same solution for 1–3 days. Brain was sectioned at 30 μm thickness on cryotome at 22°C. Serial sections were then processed for AChE staining described by Hedreen. JC et al,1985,25 Suitable controls were also maintained.

Results

Optic tectum (OT) in presently studied specimens, which constitutes the dorsal part of mesencephalon, is a bilobed structure. This is composed of six stratified zones of differently shaped and differently sized neurons. From the ependyma to the outer surface, the following strata are considered. The stratum periventriculare (SPV) in the inner region, the stratum album central (SAC), the Stratum griseum centrale (SGC) the stratum fibrosus et griseum superficiale (SFGS), the stratum opticum (SO), and the stratum margnale (SM) (Figs. 1, 2).

In Channa punctatus the outermost SM comprises two sub-zones based on AChE intensity the outer most sub-zone exhibited moderate reaction while the inner sub-zone showed intense activity. Next to SM is the SO which is wide in comparison to its adjacent zones. This layer demonstrated strong activity in the central zone while moderate reaction in the outer and inner sub-zones, SFGS showed very strong activity almost in all parts. In contrast SGC showed mild activity for AChE preparations while next to SGC, SAC exhibited no reaction, however few innervations of AChE positive somata of adjacent layers are present in SGC and SAC both. Interestingly the inner most SPV which comprises large sized somata demonstrated very high intensity (Fig. 1).

In the optic tectum of Heteropneustes fossilis, the distribution of AChE is less complex and does not correspond so precisely to histologic lamination of the nervous system. Only five zones are apparent in the present investigation. The outer most SM and next to SM, SO demonstrated intense activity while SFGS and next layer showed moderate activity. The inner most SPV, exhibited strong reaction like that of Channa punctatus (Fig. 2).

Details are given in Table 1 and Figs. 1, 2.

Discussion

The laminar distribution pattern of AChE in the optic tectum of both the species outlines the presence of cholinergic
synapses and uneven distribution of cholinergic, cholinoceptive and non-cholinergic neurons. Cells within the SPV were found to be AChE positive and similar results have been obtained in previously studied species.26-28 Thus this receptive layer appears to be a cholinergic area. Retinal afferents discharge in this layer and many of these synapses are probably cholinergic. The intense activity observed in SO points out the possible function of the most superficial neurons in OT. Dendritic branches of these marginal neurons ramify in the SO and SM and receive many synapses at this level.29, 30

SM contains fine non myelinated axons in abundance which synapse on spines of thick dendrites of pyramidal cells of SO in teleosts.30 Our study thus suggests that SM and SO have cholinergic synapses in abundance, though cholinergic somata may be comparatively less in number. This investigation is also in consonance with previous report31 which suggested that unmyelinated nerves have higher concentration of AChE than myelinated nerves.

In SFGS, characteristic terminals belonging to optic nerve are large and irregular having pale mitochondria and abundant round vesicles as suggested by EM study.32 We therefore suggest that the difference in the intensity of AChE in this layer in the currently studied specimens may be due to irregularity of optic nerve terminals. SAC and SGC layers showing mild or negative reactions are non-cholinergic which may have some cholinergic innervations of the dendrites and axons of other layers. The other ultrastructural analyses on AChE in the optic tectum of the gold fish and cat fish revealed that AChE is synthesised by all neuronal types present in layers. The fine localization of AChE is the result of its synthesis in cell bodies, its storage and transport along dendrites and its release in extra cellular space. The difference in AChE localization between two teleosts examined, mainly derive from differential enzyme release in corresponding layers.

Recently, certain non-classical functions of AChE2-4 have been elucidated that can be independent of its role in hydrolysing acetylcholine. AChE can facilitate neurite growth,2 it also acts as neuronal adhaisan protein3 and can degrade few neuropeptides as well.4 These functions explain the very wide spread staining observed in different layers which may be non-cholinergic.

Thus the essence of present discussion is that highly intense nuclei/strata may be cholinergic or cholinoceptive while moderately stained layers may be cholinoceptive merely. The mild or negatively stained layers are non cholinergic and in totality such areas are helping in the transmission of nerve impulses and playing roles in physiological and metabolic processes.

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References

1. Soreq H and Seidman S. Acetylcholinesterase: New roles for an old actor. Nat. Rev. Neurosci. 2001; 2: 294–302.
2. Downes GB and Granto M. Acetylcholinesterase function is dispensable for sensory neurite growth but is critical for neuromuscular synapse stability. Dev Biol. 2004; 70: 232–245.
3. Silman I and Sussman JL. Acetylcholinesterase, classical and non-classical functions and pharmacology. Curr Opin Pharmacol. 2005; 5: 293–302.
4. Chubb IW, Hodgson, AJ, White CH. Acetylcholinsterase hydrolyses substance P. Neuroscience. 1980; 5: 2065–72.
5. Shute CCD and Lewis PR. Cholinesterase containing system of the brain of the rat. Nature. 1963; 199: 1160–1164.
6. Krijnkev K and Silver A. The development of acetylcholinester AChE, subunit and playing roles in physiological and metabolic processes.

Table 1: AChE intensity in different layers/strata of Optic Tectum of both the fish species

| Sl. No. | Layers/Strata          | Abbreviations | AChE Activity |
|--------|------------------------|---------------|---------------|
|        |                        |               | Channa | Heteropneustes |
| 1.     | Stratum periventriculare | SPV           | +++   | +++         |
| 2.     | Stratum album centrale  | SAC           | - -   | + -         |
| 3.     | Stratum grisium centrale | SGC         | + -   | + -         |
| 4.     | Stratum fibrosum et grisium superficiale | SFGS | +++   | + -         |
| 5.     | Stratum opticum        | SO            | ++    | +           |
| 6.     | Stratum marginalae     | SM            | ++    | +           |

**Notation:**

- Very Intense: +++
- Intense: ++
- Moderate/mild: + -
- Negative: - -

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References

1. Soreq H and Seidman S. Acetylcholinesterase: New roles for an old actor. Nat. Rev. Neurosci. 2001; 2: 294–302.
2. Downes GB and Granto M. Acetylcholinesterase function is dispensable for sensory neurite growth but is critical for neuromuscular synapse stability. Dev Biol. 2004; 70: 232–245.
3. Silman I and Sussman JL. Acetylcholinesterase, classical and non-classical functions and pharmacology. Curr Opin Pharmacol. 2005; 5: 293–302.
4. Chubb IW, Hodgson, AJ, White CH. Acetylcholinesterase hydrolyses substance P. Neuroscience. 1980; 5: 2065–72.
5. Shute CCD and Lewis PR. Cholinesterase containing system of the brain of the rat. Nature. 1963; 199: 1160–1164.
8. Ishii T and Friede RL. A comparative histochemical mapping of the distribution of acetylcholinesterase and nicotinamide adenine-di-nucleotide-diphorase activities in the human brain. Inter Rev Neurobiol. 1967; 10: 231–275.

9. Bhatt DK and Tewari HB. Histochemical mapping of acetylcholinesterase and butyrylcholinesterase in the medulla oblongata and pons of squirrel. J Neurosci Res. 1978; 10: 231–275.

10. Giris M. Acetylcholinesterase enzyme localization in the amygdala: A comparative histochemical and ultra-structural study. Acta Anat. 1980; 106(2): 192–202.

11. Whittaker VD. The specificity of pigeon brain acetylcholinesterase. Biochem J. 1953; 45: 660–664.

12. Cavanagh JB and Holland P. Cholinesterase in the chicken nervous system. Nature. 1961; 190: 735–736.

13. Zuschratter W and Scheich H. Distribution of choline-acetyltransferase and acetylcholinesterase in the vocal motor system of zebra finch (*Taeiniopygia guttata*). Brain Res. 1990; 513: 93–201.

14. Cooksen KK, Hall WS, Heaton JT et al. Distribution of choline-acetyltransferase and acetylcholinesterase in vocal control nuclei of the budgerigar (*Melopsittacus undulatus*). J Comp Neurol. 1996; 369: 220–235.

15. Sethi JS and Tewari HB. Histoenzymological mapping of acetylcholinesterase in the diencephalon and mesencephalon of *Uromastix hardwickii*. J Hirnforsch. 1976; 17(4): 335–349.

16. Sethi JS and Tewari HB. Histochemical mapping of acetylcholinesterase in the cerebral hemispheres of *Uromastix hardwickii*. Cell and Molec Biol. 1977; 22: 263–275.

17. Srivastava UC and Tripathi A. Histochemical localization of acetylcholinesterase in the cerebellum and optic tectum of four freshwater teleasts. J Histoch. 1975; 45: 279–288.

18. Contestabile A. Histochemical study on the distribution of some enzyme activities in the vaginal and facial lobes of the gold fish, *carassius auratus*. Histochemistry. 1975; 44: 123–132.

19. Sood PP, Sinha SP. A comparative histochemical study of alkaline phosphatase and acetylcholinesterase in the hind brain of *Channa punctatus* and *Heteropneustis fossilis*. Folia Histochem cytochem. (Krakow) 1983; 21(2): 107–114.

20. Meek J, Schellart NA. A Golgi study of gold fish optic tectum. J Comp Neurol. 1978; 182: 89–122.

21. Castro A, Becerra M, Manso MJ, et al. Calretinin immunoreactivity in the brain of the zebra fish *Danio rerio*: Distribution and comparison with some neuropeptides and neurotransmitter synthesizing enzymes. II Mid brain, hind brain and rostral spinal cord. J Comp Neurol. 2006; 494: 792–814.

22. Hedreen JC, Bacon SJ, Price DL. A modified histochemical technique to visualize acetylcholinesterase containing axon. J Histoch Chem. 1985; 33: 134–140.

23. Northcut RG and Butler AB. The diencephalon and optic tectum of the longnose gar, *Lepisosteus osseus* (L): Cytoarchitectonics and distribution of acetylcholinesterase. Brain Behav Evol. 1993; 41: 57–81.

24. Wullimann MF, Meer DL. Phylogeny of putative cholinergic visual pathways to the hypothalamus in teleost fishes. Brain Behav Evol. 1990; 36: 14–29.

25. Butter AB and Saidal WM. Retinal projections in the freshwater butter-fly fish *Pantodon buchholzi* (Osteoglossoidei), I Cytoarchitectonic analysis and primary visual pathways. Brain Behav Evol. 1991; 38: 127–153.

26. Laufar M and Vanegas H. The optic tectum of a perciform teleost I, General configuration and cytoarchitecture. J Comp Neurol. 1974; 154: 43–60.

27. Nachmensohn D. Chemical and molecular basis of nerve activity. Physiologically significant features of acetylcholinesterase. Academic Press, New York and London1959.

28. Villani L, Clani F, Contestabile A. Electron Microscope histochemistry of acetylcholinesterase distribution in the optic tectum of teleost. J Hirnforsch. 1979; 20(5): 539–551.