Histoenzymological Mapping of Acid and Alkaline Phosphatase in the Telencephalon of Hillstream Teleost *Barilius bendelisis* (Hamilton)

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The paper describes for the first time the distribution of acid and alkaline phosphatase in the forebrain of a hillstream teleost *Barilius bendelisis* (Hamilton). The intensity of alkaline phosphatase is not so pronounced as that of acid phosphatase. The nuclear areas show more intensive activity of acid phosphatase than fibre tracts whereas the case is reverse with alkaline phosphatase.

The brain is metabolically organized in ways considerably different than the other organs. The understanding of functional significance of metabolic differences have generally been deduced from the studies on the distribution of metabolites (including enzymes) in brain and many of such information come from the studies at intracellular level. Another approach for such studies, i.e., the distribution at topographic level, is also important because it provides an opportunity to measure and compare the relative sites and concentration of metabolites in different cytoarchitectural areas of brain with respect to neuroanatomical structures. The literature available provides very little information of this sort on the the brain of fish. Therefore, an attempt has been made to study the regional localization and functional significance of acid and alkaline phosphatase in the telencephalon of a hillstream teleost *Barilius bendelisis*.

MATERIALS AND METHODS

Live specimens of *B. bendelisis* were procured from Khandagad, a small springfed tributary of the river Alaknanda in Garhwal Himalaya. These were decapitated without
using any anaesthesia, their complete and intact brains were dissected out and fixed in 70% chilled neutral formalin at 4o C. for 12 hours and embedded in gelatin. Twenty five micron thick cross sections, cut on freezing microtome, were again washed thoroughly in ice cold distilled water and processed for acid and alkaline phosphatase, following the standard methods.

Proper controls were also made simultaneously by incubating the sections in substrate free media. Various nuclear areas and fibre tracts were identified with the help of Adriëns Kappers, Singh and Kuhlenbeck.

RESULTS

Nuclear areas—In the precommissural part of basal region, olfactory tubercle, the prepiriform area and polymorph layer show a moderate reaction for both acid (Fig. 1) and alkaline phosphatase (Fig. 2). But the nuclei of postcommissural basal region preoptic part—the nucleus preopticus magnocellularis, nucleus preopticus parvocellularis and nucleus preopticus parvocellularis pars recessi, display a moderate reaction for acid phosphatase (Figs 5-6) and strong reaction for alkaline phosphatase (Figs. 10-11). Similarly, medial and lateral septal nuclear areas of telencephalic septal region are moderate for acid phosphatase activity (Fig. 1) and strong for alkaline phosphatase reaction (Figs. 7-8). The nuclear areas of dorsomedial region of telencephalon—the primordial hippocampus and primordial hippocampus pars infimbrialis, exhibit a moderate and strong reaction for acid (Figs 1,2,5) and alkaline phosphatase (Figs. 7-9,11) respectively. But the reaction for both of these hydrolytic enzymes seem to be inconsistent in the bed nucleus of stria terminalis and bed nucleus of anterior commissura.

The part of piriform area, very close to pars distalis and median diagonal sulcus, is especially strong in alkaline phosphatase concentration (Fig. 7), whereas other nuclei of the intermediate lobar region—pars dorsalis, caudoventral and rostroventral components of striate body and nucleus entopeduncularis,—lateral lobar region—piriform area, dorsolateral and ventromedial nuclei of amygdaloid complex, display a moderate activity for both acid (Figs. 1,2,5) and alkaline phosphatase (Figs. 7-9,11). Fibre tracts—Among the tracts related to the olfactory tubercle—tractus olfactorius medialis pars medialis, tractus olfactorius medialis pars medialis and tractus olfactorius lateralis show strong reaction for acid phosphatase (Figs. 1,2) and moderate reaction for alkaline phosphatase (Fig. 8). All the components of commissura anterior—the commissura olfactorii internuclearis and commissura olfactoria interbilbaris, exhibit a strong reaction for acid phosphatase except in commissura
hippocampii which shows a moderate presence of similar reaction (Figs. 2-4), but there is a poor activity of alkaline phosphatase in all the component parts of the commissura (Figs.

Figs. 1-6 Acid phosphatase activity:

Figs. 1 & 2. Cross sections passing through rostral to and through commissura anterior X 120.

Figs. 3 & 4. Parts of fig. 2 magnified X300.
9-10). In the lateral forebrain bundles—the tractus olfacto hypothalamo et hypothalamicus olfactorius lateralis and tractus striato-thalamicus, the reaction is strong and moderate for acid (Figs 2-6) and alkaline phosphatase (Figs 9, 11) respectively. There is strong reaction for acid phosphatase in the medial forebrain bundles—the tractus olfacto hypothalamo et hypothalamicus olfactorius medialis (Fig. 4), but inconsistent when observed for alkaline phosphatase. The observations on the regional distribution are summarised in the Table 1.

Fig. 5. Cross section passing through caudal to commissura anterior X 120.

Fig. 6. Part of fig. 5 magnified X 300.

Figs. 7-11. Alkaline phosphatase activity:

Figs. 7 & 8. Cross sections passing through rostral to commissura anterior X 120.
Fig 9. Cross section passing through commissura anterior X 120.
Fig. 10. Part of fig. 9 magnified X300.
Fig. 11. Cross section passing through caudal to commissura anterior X 120.

Abbreviations used: AMYD—dorsolateral nucleus of amygdaloid complex; AMYV—ventromedial nucleus of amygdaloid complex; CAT* commissura anterior; CH—commissura hippocampi; LFB—lateral forebrain bundles; MDS—median diagonal sulcus; MFB—medial forebrain bundles; NET—nucleus entopeduncularis; NPM—nucleus preopticus magnocellularis; NPP—nucleus preopticus parvo cellularis; NPPR—nucleus preopticus parvo cellularis pars rocessi; NSL—lateral septal nucleus; NSM—medial septal nucleus; PD—pars dorsalis; PH—primordial hippocampus; PHF—primordial hippocampus pars fimbrialis; PIR—piriform area; POLT—polymorph layer of olfactory tubercle; PRE—prepiriform area of olfactory tubercle; PSC—caudoventral component of
Table 1: Distribution of acid and alkaline phosphatase in the forebrain of *B. bendelisis*.

<table>
<thead>
<tr>
<th>Name of nuclei/fibre tract</th>
<th>Acid phosphatase</th>
<th>Alkaline phosphatase</th>
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<tbody>
<tr>
<td><strong>Nuclei</strong></td>
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<tr>
<td>POLT</td>
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<td>1</td>
</tr>
<tr>
<td>PRE</td>
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<td><strong>Fibre tracts</strong></td>
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<tr>
<td>CAT</td>
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<tr>
<td>CH</td>
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Activities are expressed by: +++ strong; ++ moderate; + poor or mild, ± inconsistent.
DISCUSSION

There are a number of variations as regards the degree of intensity of acid phosphatase.8 22 The presence of acid phosphatase in the neurons and fibres of telencephalon of B. bendelisis is in conformity with the earlier reports8 22 whereas the fibre tracts (axonic fibres) show higher levels of acid phosphatase reaction, contrary to these observations:

Lavelle et al.10 attributed a direct correlation between the acid phosphatase activity and Nissl substance in nerve cells which account for the reaction observed in neurotic cell bodies of various nuclear areas during the present investigations. The amount of acid phosphatase seems also to be associated with the lysosomes present in the neurotic bodies.11

The level of acid phosphatase activity nerve cell perikarya and in neuropil appears to be inversely proportional. Much of the activity is found in the neuropil of nuclei which show little activity in perikarya and vice-versa. This inverse relationship appears to minimize the variations in activity for different nuclei despite contrasting cytological patterns of enzyme distribution.

The strong activity of acid phosphatase in fibre tracts may be attributed to its association with neurokeratin13 14 and other lipid substances in myelin sheath of fibres, contrary to the observation in higher vertebrates. Later studies15 16 19-21, on a number of vertebrate species correlated the enzymatic activity with physiological state. Broadwell24 suggested that the acid hydrolase containing organelles in the neurons are not static or rigidly defined system.

Earlier investigations on higher vertebrates1 23 24 have demonstrated predominant concentration of alkaline phosphatase in the blood vessels and it may also be located in the endothelial cells25. In the forebrain of B. bendelisis, the nuclear areas show either moderate or strong alkaline phosphatase reaction while fibre connections display poor to moderate reaction. This is due to differential pattern of blood capillaries supplying to nuclear areas and fibre tracts. In the light of earlier observations it is assumed that in the neuropil of nuclear areas, strongly positive alkaline phosphatase activity may be playing a role in transport of selective metabolites and thus playing important role in blood-brain barrier and also in maintenance of the constancy of brain environment for normal physiological functions.

Friede22 reported the possibility of inverse relationship of alkaline phosphatase activity in neuropil and blood vessels, i.e., the areas which have high levels of alkaline phosphatase
show low activity in capillaries and vice-versa. This was further confirmed by the studies on other vertebrates\textsuperscript{17} \textsuperscript{18} \textsuperscript{25}. The present study also support the contention.

The study also demonstrates a great deal of regional variation in the distribution of alkaline phosphatase as compared to acid phosphatase as evident from Table 1, i.e. strong to moderately positive alkaline phosphatase reaction in nuclear areas and moderate to poor activity in the fibre tracts. Acid phosphatase reaction show much less variation.

Thus, the reaction of alkaline phosphatase in grey and white matter and cytological distribution of this enzyme differs fundamentally from that of acid phosphatase because phosphatase is usually localized along the plasma membrane and other cytembranes\textsuperscript{27}.

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REFERENCES