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Physiology

HISTOCHEMICAL MAPPING OF LIPIDS AND PROTEINS IN THE MESENCEPHALIC NUCLEI AND FIBRE TRACTS OF HILLSTREAM TELEOST *BARILIUS BENDELISIS* (HAM.)

HISTOCHEMICZNE ROZMIESZCZENIE LIPIDÓW I BIAŁEK W JĄDRACH ŚRÓDMÓZGOWIA I WŁÓKNACH *BARILIUS BENDELISIS* (HAM.)

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The observations on histochemical localization of lipids including phospholipids and proteins have been made in the nuclear areas and fibre tracts of the mesencephalon of a hillstream minor carp *Barilius bendelisis* (Hamilton). The different layer of optic tectum exhibit moderate to intense contents of lipids and phospholipids whereas protein concentration varies from negative to intense. Most of the nuclei of mesencephalic tegmentum show moderate concentration of Sudan Black B and acid haematin positive materials and similarly of marcury bromophenol blue positive substances. Practically all the fibre connections have either intense or strong staining of Sudan Black B and acid haematin tests. But the presence of proteins in some tracts is either negligible or poor or inconsistent. In nuclear areas, intracytoplasmic membranous organelles contribute to the major fraction of lipids whereas it is myelin of the sheaths of axonic fibres.

INTRODUCTION

The histomorphology of the mesencephalon of Indian teleosts has been worked out by a number of workers with the help of classical histological techniques (Singh and Khanna

1972; Sharma 1983; Kaur 1985) but the studies on the localization of various metabolites in different parts of the central nervous system are fragmentary and meagre to ascertain the peculiarities of metabolism of nervous tissues of these primarily aquatic but highly specialized vertebrate, however, a number of references are available on such aspects of higher vertebrates. Therefore, the present work on regional localization of lipids including phospholipids and proteins in the nuclei and fibre tracts of the mesencephalon of *Barilius bendelisis,* a common representative of hillstream teleosts in Garhwal Himalaya, intends to be an initial step in this fascinating direction.

MATHERIAL AND METHOD

Live specimens of *B. bendelisis* procured from Khandagad, a small springfed tributary of river Alaknanda in Garhwal Himalaya (30° 11′ N, 78°46′ E, 560 MSL) were brought to the laboratory and acclimatized for three days. Their complete and intact brains were dissected out and immediately fixed in calcium-formol and 10% neutral formalin for 18 hours at room temperature. The calcium-formol fixed brains were post-chromated and embedded in gelatin 15 μm thick cross sections passing through mesencephalon sectioned on cyrostat were processed for Sudan B (SBB) and acid heamatin (AH) tests for lipids and phospholipids respectively (Baker 1946, 1956; Cain, 1947 of. Pearse 1968). Proper controls were also run simultaneously as suggested by Adams and Bayliss (1962). 10 μm thick cross sections of formalin fixed paraffin blocks were treated for mercury bromophenolblue (Hg BPB) procedure for protein (Bonhag 1955 cf. Pearse 1968). Various histobiological structures were identified with the help of Ariens Kappers et al. 1936 and Kuhlenbeck 1975.

RESULTS

NUCLEAR AREAS –

Like other teleosts, the mesencephalon in *B.bendelisis* is further divisible into the optic tectum (OT), torus longitudinalis (TL) and tegmentum.

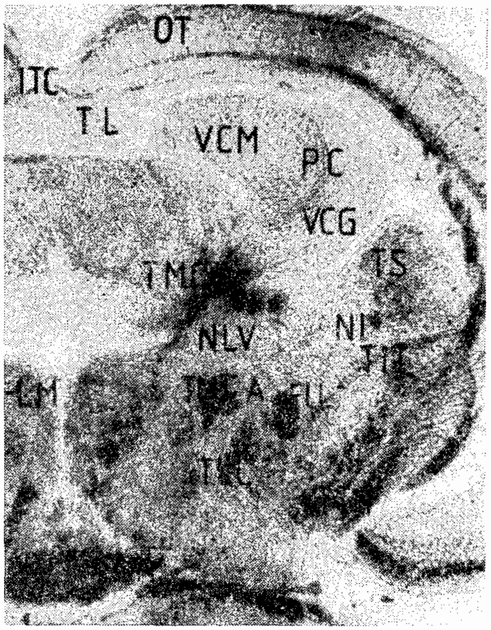
Optic tectum – The presence of SBB and AH positive substances were essentially similar if different layer of optic tectum (Fig. 1,3). The layers which are fibrous in cytoarchitecture ahowed rich concentration of lipids including phospholipids as compared to the layers of neuronic nature. Moreover, the intensity of sudanophilic substances was more than AH positive materials in various layers. The retinic fibrous layer of stratum opticum (STOR), stratum album centrale (STAC) exhibited intense concentration of lipids and phospholipids both. The marginal fibrous layer of stratum opticum (STOM), stratum fibrosum-et-griseum superficiale (STFGS) and stratum griseum centrale (STGC) showed strong staining of SBB and moderate staining of AH tests. Whereas stratum

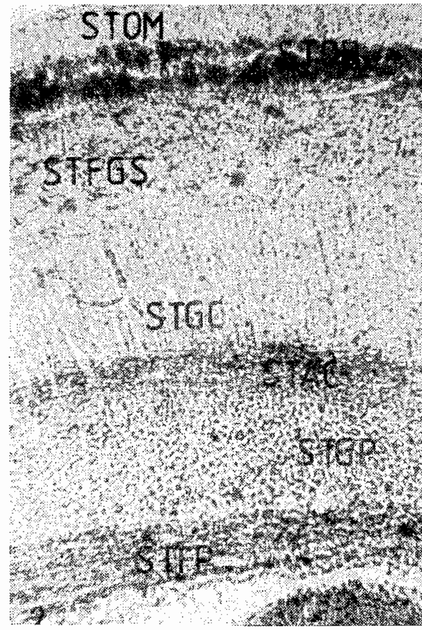
Table 1

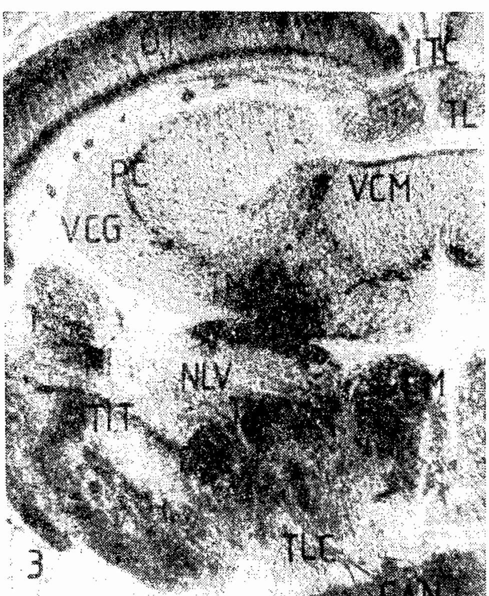
Distribution of SBB, AH and HgBPB positive substances in he the mesencephalon of *B. bendelisis*

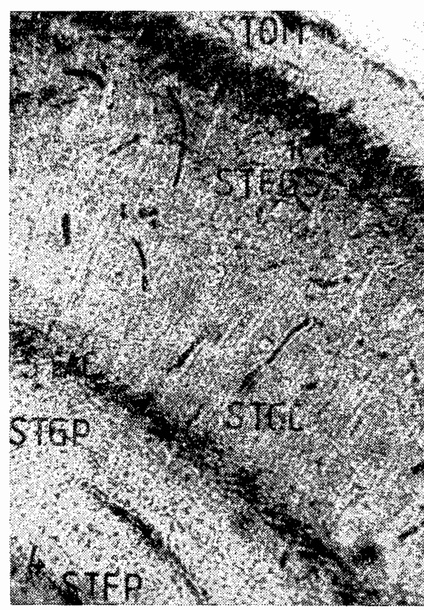
Name of nuclei/ fibre tract	SBB	AH	HgBPB
NUCLEAR AREASOPTIC TECTUM			
STOM	+++	++	+++
STOR	++++	++++	0
STFGS	+++	++	+++
STGC	+++	++	+++
STAC	++++	++++	++++
STGP	++	++	0
STFP	+++	+++	+++
TORUS LONGITUDINALIS	+++	+++	+++
TEGMENTUM			
TS	++	++	++
NLV	+	+	++
NIP	+	0	++
NI	++	++	++
NPM	++	++	++
NRM	++	++	++
ND	++	++	++
NT	++	++	++
FIBRE TRACTS–			
FMNO	++	+++	±
TTT	+++	+++	±
TSTM	+++	+++	±
TIT	+++	+++	0
CAN	++++	++++	+
TML	+++	+++	±
TLC	+++	+++	+
LCT	+++	+++	+
ITC	++++	++++	+
TMCA	++++	++++	+
TMCP	++++	++++	+
FLM	++++	++++	+
FLL	++++	++++	+
TALL	+++	+++	±
TD	+++	+++	±
TT	+++	+++	±

(concentrations are shown as ++++ – intense, +++ – strong, ++ – moderate, + – poor or weak, 0 – negative or negligible, ± – inconsistent or not clear) Abbreviations as in the text.





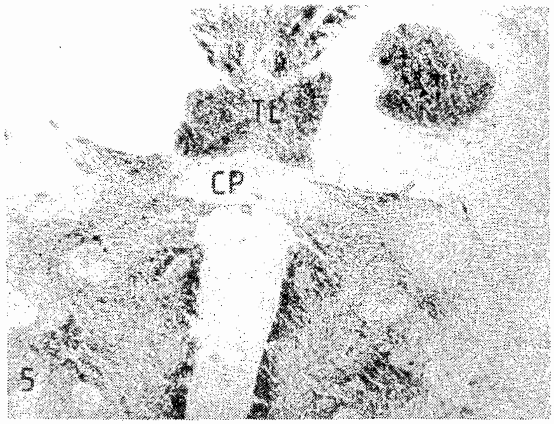


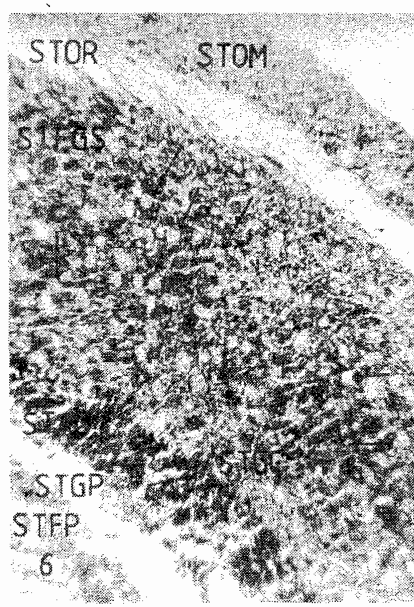


(Figs 1–2) SBB preparations, Figs 3–4 AH preparations and Figs 4–7 HgBPB preparations)

Figs 1,3 – Cross section (cs) of brain through mesencephalon (note intense lipids and phospholipid concentration in ITC, TMCA, TMCP, FLL, FLM, CAN etc, and strong staining in TL, TS, TLC etc.) x 120)

Figs 2, 4 – cs of brain through optic tectum, intense staining in STOR, STAC x 600





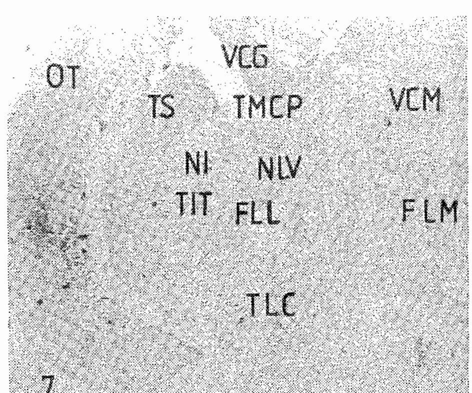


Fig. 5 – cs through the begining of TL which exhibits strong protein contents x 120

Fig. 6.– cs of optic tectum showing optically empty areas of interiors of axonic fibres (arrows) x600

Fig. 7. cs through caudal mesencephalon exhibiting weak staining in TMCP, TLC, FLM etc. x120

griseum periventriculare (STGP) had moderate and stratum fibrosum periventriculare (STEP), exhibited strong concentration of both of these substances.

The results of HgBPB preparations (Fig. 6, Table 1) revealed intense contents of proteins in STAC; strong in STOM, STFGS and STGC; poor in STFP and negligible in STOR and STGP. In the middle layers (STGS and STGC), the axonic fibres in sections (arrows) appeared as optically empty entities. Torus longitudinalis (TL) – All the cellular components of TL especially those lying close to posterior commissure and medial of the organ showed strong concentration of lipids and phospholipids (Fig. 2, 4) whereas practically all the cellular components had strong staining of HgBPB test (Fig. 5).

Tegmentum – Most of the nuclear in mesencephalic tegmentum of *B. bendelisis* showed moderate presence of SBB and AH positive tests especially in torus semicircularis (TS), nucleus isthmi (NI), nucleus profundus mesencephali (NPM), nucleus reticularis mesencephali (NRM), nucleus, occulomotorius (ND) and nucleus trochlearis (NT) whereas the concentration of these substances was weak in nucleus interpeduncularis (NIP) (Fig. 1,3). All these nuclear areas were observed to be with moderate staining, in HgBPB treatment (Fig. 7).

Besides, ventricle of mesencephalon also gives the space to the valvula cerebelli, a rostral extension of cerebellum. It is composed of granular and molecular layers (VCM, VCG) sandwitched by the Purkinje cells layer (PC). The concentration of SBB and AH positive substances was moderate in VCM and VCG but intense in PC (Figs 1, 3).

FIBRE TRACTS –

The optic tectum, torus longitudinalis and mesencephalic tegmentum in *B. bandelisis* are connected with each other and other parts of the brain by a number of afferent and efferent fibre tracts. The concentration of SBB and AH positive materials in these were either intense or strong (Figs 1, 3). The tracts witj strong SBB and AH staining are fasciculus medialis nervi optici (FMNO), tractus toro tectalis (TTT), tractus spino tectalis-et-mesencephalicus (TSTM), tractus mesencephalo lobaris (TML), tractus lobo cerebellaris (TLC), laminar commissuralis tecti (LCT), tractus acoustico lateralis lemniscus (TALL), tractus occulomotorius (TO) and tractus trochlearis (TT) while the tracts with intense staining are commissura ansulata (CAN, tractus tecto bulbaris ventralis rectus and cruciatus), intertectal commissure (ITC), tractus mesencephalo cerebellaris anterior and posterior (TMCA, TMCP), fasciculus longitudinalis medialis and lateralis (FLM, FLL).

As regards the results of HgBPB preparations (Fig. 7) in fibre tracts, the staining was poor in most of the tracts except in TIT with negligible staining and FMNO, TTT, TSTM, TML, TALL, TO and TT in which the results were not very clear.

DISCUSSION

Lipids (phospholipids)

Much of the work on lipid neurochemistry deals with higher vertebrates (Alsterberg 1941a, 1941b, 1945, 1948; Malhotra 1960; Singh 1964) and, in fact, there are a few references regarding the histochemical distribution of lipids in various neuroanatomical structues of fish brain particularly Indian teleosts. The observations on lipid distribution in *Notopterus notopterus Puntius ticto* (Saxana and Johri 1970) *Barilius bendelisis* and *Barilius vagra* (Tandon and Sukheja 1974) and some other teleosts (Sharma 1983; Kaur 1985) reveal deep staining with SBB and AH tests in fibres and commissures; the neuronic areas were observed with moderate staining. During present investigations, the findings are in conformity with the studies made by earlier workers, i.e., in moderate to intense concentrations in different layers of optic tectum depending on their neuronic or fibrous or comoposite cytoarchitecture; moderate lipid contents in most of the nuclei of mesencephalic tegmentum (except in NIP and nucleus laterali valvulae NLV), strong staining in TL while intense to strong concentration in the tracts and commissures.

In nuclear areas, it is the neuropil of grey matter, membranes of intracytoplasmic organelles especially microsomal and mitochondrial membranes contributing to the major part of lipid and phospholipids both (Petersen and Schou 1955; Kreps et al. 1964; Friede 1966). In cold blooded vertebrates including fishes, Sharma (1968) described the histochemical nature of lipid bodies in neurons comprising the lipids, lipoproteins, pigments and some basophilia like Nissl substances. Myelin sheath of axons (consttuting of fibre tracts and fibrous layers of optic tectum) consisting of concentratic and alternate lipid-protein envelops accounts for dominant fraction of total lipid and phospholipids in addition to the generally occurring intra-axonal organelles and inclusions present in the axoplasm. The concentric lipidprotein envelops of myelin and cytomembrans of intimately interlacing processes of neurons and glia abound make the lipids and phospholipids (Fierde 1966).

In the diencephalon of freshewater turtle *Lissemys punctata granosa*, Gautam and Sood (1982) obtained essentially the similar results of phospholipids, β-glucouronidase and β-galactosidase preparations, therefore they concluded that these two enzymes play a significant role in lipid metabolism. *B. bendelisis* is a surface dweller and a sight feeder with the senses of vision and orientation being vitally important in the integration of overall behavioural pattern and body responses. In such a subject, lipid metabolism in optic tectum (especially STOR, STFGS, STAC) and tracts related to photofunctions appears to be dependent upon the photoperiod, intesity and quality of light determined by the depth and nature of water column (Anderson et al. 1985). Thus, the lipid metabolism and their occurrance in nuclear areas and fibre connections in fairly good concentrations probably seems to be due to increased rate of lipid turnover and/or enhanced biosynthesis in microsomes (Freysz et al. 1985) and also lipid dependent changes in the transport of various metabolites.

Proteins

The observations of HgBPB preparations indicate good concentration in STOM, STFGS, STGC, STAC, STFP and practically all nuclear areas of mesencephalic tegmentum while fibre tracts exhibit either negative or poor staining or the results are not very clear. In nuclear areas, protein contents are particularly higher in nuclear membrane and cytoplasm of neurons. The poor protein concentration of various fibre tracts is due to the myelination of axonic fibres in which lipid-protein alternate and concentric envelop formations lipids predominate. Interestingly, in the middle layers of optic tectum, the axonic fibres in the sections look as optically empty spaces (arrows). Thus, whether the protein have been localized in these and also in fibre tracts, it is the part of myelin sheath. Benowitz and Lewis (1983) and Benowitz et al. (1983) observed in detail the changes in protein moities during regeneration and degeneration of gold fish optic nerve and both qualitative and quantitative changes in protein moities and intra-axonal transportation along axons have been observed (Grafstein and Froman 1980). On the basis of these studies, it is possible that proteinaceous content of axoplasm (and hence tracts etc) may have respondedn quickly to sudden and abrupt dissection of brain rendering them more soluble. Therefore, the tracts and interiors of axonic fibres especially in the middle layer of optic tectum (shown by arrows in Fig. 6) are poor or negative for protein conents probably because of the fact that there is either leaching out of proteinous substances during routine process of fixation, embedding and following HgBPB treatment or intra-axonal proteins may not be fixed by conventional methods and need other specific techniques.

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HISTOCHEMICZNE ROZMIESZCZENIE LIPIDÓW I BIAŁEK W JĄDRACH ŚRÓDMÓZGOWIA I WŁÓKNACH *BARILIUS BENDELISIS* (HAM.)

STRESZCZENIE

Przeprowadzono obserwacje na temat chistochemicznej lokalizacji lipidów włączając w to fosfolipidy oraz białka proste w rejonach jąder i włókien mesencephalon – śródmózgowia karpia *Barilius bendelisis* (Ham.). Różne warstwy optic tectum moderują zawartość lipidów i fosfolipidów podczas gdy koncentracja protein zmienia się.

Większość jąder z mesencephalic tegmentum wykazuje umiarkowane koncentracje Sudanu B, kwaśnej hematyny i podobnie błękitu bromofenolowego.

Praktycznie całe włókniste połączenia mają intensywne lub silne wybarwienie w testach z Sudanem B i kwaśną hematyną. Obecność protein w niektórych przewodach jest znikoma lub mała.

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