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HISTOENZYMOLOGICAL LOCALIZATION OF GLYCEROL 3 PHOSPHATE DEHYDROGENASE AND SUCCINATE DEHYDROGENASE IN THE MESENCEPHALON OF A MINOR CARP BARILIUS BENDELISIS (HAMILTON)

ROZMIESZCZENIE I AKTYWNOŚĆ DEHYDROGENAZ W ŚRÓDMÓZGOWIU *BARILIUS BENDELISIS* (HAMILTON)

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This contribution deals with the studies on histoenzymological localization of two oxtdative enzymes, glycerol 3 phosphate dehydrogenase (G 3PD; EC 1.1.1.8) and succinate dehydrogenase (SD; EC 1.3.99.1) in the mesencephalic nuclei and fibre tracts of a minor carp *Barilius bendelisis*. The nuclear areas exhibit weak to moderate concentration. High activities have been observed in the retinic fibrous layer of stratum opticum (STOR), stratum album centrale (STRAC) of optic tectum and nearly all fibre tracts which also show similar pattern of activities of both dehydrogenases. In the brain of *B. bendelisis*, 63 PD appears to help in the biosynthesis and maintenance of myelin; glucose seems to be chief energy molecule being metabolised first anaerobically in cytoplasm and then aerobically in mitochondria. Pentose shunt pathway also possibly exists to supplement the main energy cycle during exigencies.

INTRODUCTION

Among vertebrates, teleost mesencephalon is rather peculiar because its ventricle provides enough space to be occupied by valuvula cerebelli, the anterior extension of cerebellum. It acts as the control room of a number of motor and sensory functions. The histo-morphological structures of fish mesencephalon are exhaustively known (Ariens

Kappers et. al., 1936; Singh, Khanna, 1972; Kuhlenbeck, 1975). Very litlle information are available on the localization and functional role of various metabolites particularly oxidative enzymes in fish brain, however, many references appeared on histochemical and biochemical aspects in the central nervous system of higher vertebrates (Laatsch, 1962; Friede, 1966; Manocha and Shantha, 1969; Tewari and Sood, 1974; Sood and Hafiza, 1981; Iijima et al., 1984; Montz et al., 1985). To initiate such line of work, the regional localization of glycerol 3 phosphate dehydrogenase (G 3 PD, EC 1.1.1.8) and succinate dehydrogenase (SD, EC 1.3.99.1) involved in glucose metabolism have been made in the nuclear areas and fibre tracts of mesencephalon of a minor carp *Barilius bendelisis.*

MATERIAL AND METHODS

Live specimens of *B. bendelisis* were procured from Khandagad, a small tributary of river Alaknanda in Garhwal Himalaya, brought to the laboratory and acclimatized for three days. To complete and intact brains were dissected aut (5 animal of 10–12 cm length) and immediately fixed in chilled 10% neutral formalin at 4°C for 30 minutes. Twenty micron thick cross sections passing through mesencephalon were cut on cryostat and processed for G 3 PD and SD (Pearse, 1972; Kiernan, 1981). Proper controls were also made simultaneously by boiling the section in distilled water for 5 minutes before incubation. Various nuclei and fibre tracts were identified with the help of Ariens Kappers et al., 1936; Singh and Khanna, 1972; Kuhlenbeck, 1975).

RESULTS

Nuclear Areas

The mesencephalon in *B. bedelisis* is further divisible in the optic tectum, torous longitudinalis and tegmentum.

OPTIC TECTUM (TO, Figs 1,4) – The marginal fibrous layer of stratum opticum (STOM) show strongly positive activity of G 3 PD and SD. The activities of these two enzymes were observed to be intensely positive in retinic fibrous layer of optic tectum (STOR); moderate reaction in stratum fibrosum-et-griseum superficiale (STFGS), stratum griseum centrale (STGC) and weak in stratum griseum periventriculare (STGP). In rest of the layers, the activities differ, i.e., intense G 3 PD and strong SD activities in stratum album centrale (STAC); moderate G 3 PD and strong SD activities in stratum fibrosum periventriculare (STEP).

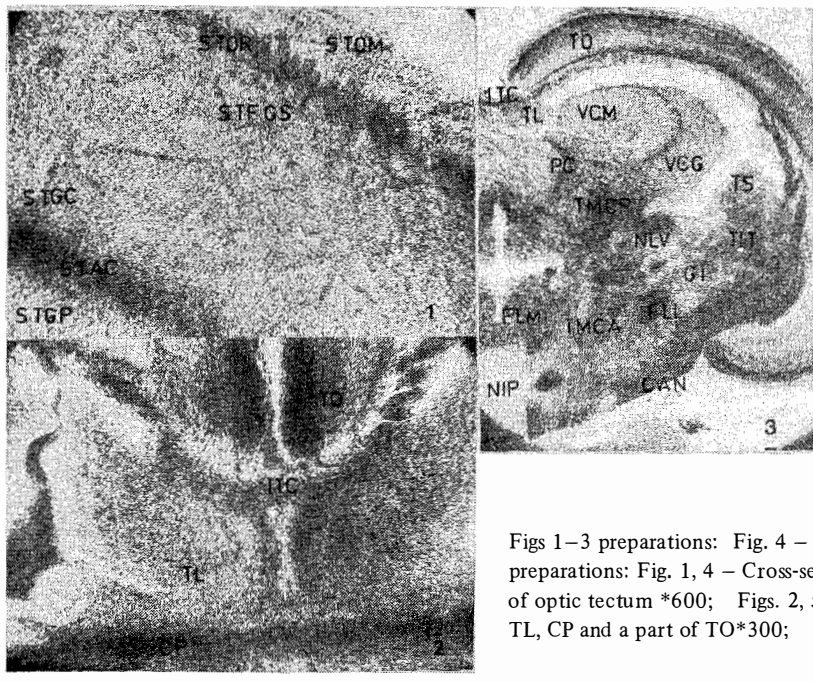
TORUS LONGITUDINALIS (TL, Figs 2,5) – The activities of both the dehydrogenases were intense in the cellular elements lying near the commissura posterior (CPT) intertectal commissure (ITC) and medial region while the cells occupying the outskirts of the organ exhibited moderate to strong activity.

Table 1

Distribution of G3PD and SD activities in the mesencephalic nuclei and fibre tracts of *B. bendelisis*

NAme of nuclei/fibre tract	G3PD	SD
Nuclear areas –		
OPTIC TECTUM –		
STOM	+++	++
STOR	++++	++++
STFGS	++	++
STGC	++	++
STAC	++++	++++
STGP	+	+
STFP	++	+++
TORUS LONGITUDINALIS (TL)	++/+++	++/+++
TEGMENTUM –		
TS	++	+++
NIP	+	+
NLV	+	+
NI	++	++
NPM	++	++
NRM	++	++
NO	±	±
NT	±	±
VCM	++	++
VCG	++	++
PC	++++	++++
Fibre tracts –		
FMNO	+++	+++
TTT	+++	+++
TSTM	+++	+++
TIT	+++	+++
CAN	++++	++++
LC T	+++	+++
ITC	+++	+++
TMCA	++++	++++
TMCP	++++	++++
FLL	++++	++++
FLM	+++	+++
TT/TO	±	±

(activities are shown as ++++ – intense, +++ – strong, ++ – moderate and + – weak or poor and ± – – not clear. Abbreviation in the text).



Figs 1–3 preparations: Fig. 4 – 6 SD preparations: Fig. 1, 4 – Cross-sections(cs) of optic tectum *600; Figs. 2, 5 – cs of TL, CP and a part of TO*300;

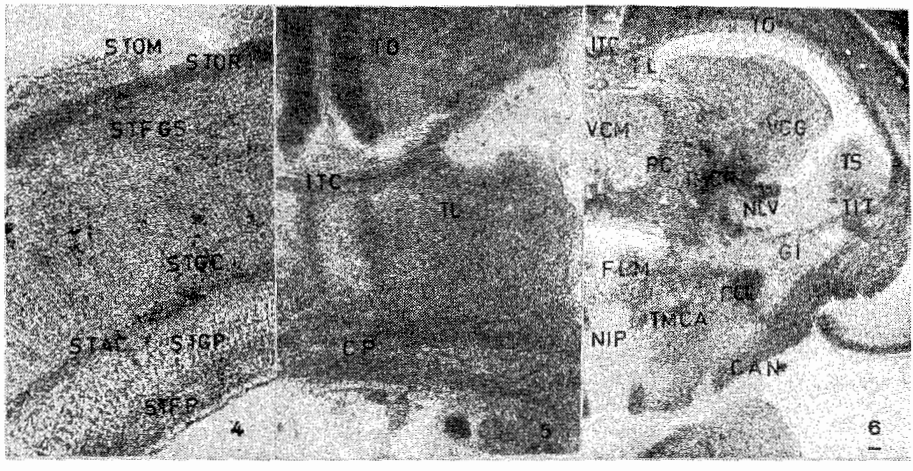


Fig. 3,6-cs of middle part of mesencephalon *120 (abbreviations as in the text)

TEGMENTUM (Figs 3,6)– Both of the deyhdrogenases were observed to be in weak concentration in the nucleus lateralis valvuli (NLV), nucleus isthmi (NI), nucleus interpeduncularis (NIP); with moderately positive activities in nucleus profunds mesencephali (NPM), nucleus reticularis mesencephali (NRM) while with strongly positive in torus semicircularis (TS). The activities were not clear in the nucleus occulomotorius and the nucleus trochlearis (NO NT).

Besides, the valvula cerebelli which is a rostral extension of cerebellum and accupy the space available in the mesencephalic ventricle; it has similar cellular configuration as that of corpus cerebelli. It exhibited moderate G 3 PD and SD activities in the molecular and granular layers (VCM; VCG) and intensely pasitive activities in Purkinje cells (PC) and their fibres.

Fibre Tracts (Figs 3, 6)

The optic tectum, torus longitudinalis and tegmentum are connected with each other and other brain parts by a number of afferent and efferent fibre tracts. G 3 PD and SD activities in these were either strongly or intensely positive. The fibre tracts which ahowed strong activity are fasciculus medialis nervi optici (FMNO), tractus toro tectalis (TTT), tractus spino tectaliset-mesencephalicus (TSTM), tractus isthmo tectalis (TIT), laminar commissuralis tecti (LCT), intertectal commissure (ITC), Tractus acoustic-lateralis lemniscus (TALL) and brachium conjunctivum (BC). The commissura ansulata (CAN, including tractus tecto bulbaris ventralis cruciatus and rectus), tractus mesencephalo-cerebellaris anterior and posterior (TMCA, TMCP), fasciculus longitudinalis lateralis and medialis (FLL, FLM) exhibited intense reaction while the activity was not clear in tractus occulomotorius and tractus trochlearis (TO, TT).

The observations are summarily recorded in Table 1. The abbreviations used for various nuclei and fibre tracts are as per convenience.

DISCUSSION

G 3 PD is an important cytoplasmic enzyme catalysing the conversion of glycerol-3-phosphate to 1–3 diphosphoglycerate during anaerobic glycolysis and possibly providing a link between glycolysis snd hexose monophosphate shunt pathwy. SD occurs in mitochrondrial materix mediating reduction of succinate into fumerate. The present study reveals almost similar degree of activities of G 3 PD and SD in different mesencephalic nuclei and fubre tracts (Table 1: implying that dehydrogenases involved in the energy metabolism follow almost identical patterns of distribution because the product of one dehydrogenase is essential pre-requisite for another step (Friede, 1966).

In spite of being an integral part of carbohydrate utilization and biosynthesis of membrane phospholipids (Laatsch, 1962) in nervous tissues, the histoenzymological and biochemical reports of G 3 PD are lacking in fish. However, it has been noted to be very active in oligodendrocytes of rat brain and pig brain (Laatsch, 1962; Cammer and Zimmerman, 1983; Montz et al., 1985). Sharma (1983), while studying the brain of four

Indian teleosts, reported in SD preparations, deep staining in neurons as compared to fibrous regions; moderate activity in the stratum opticum and STGP etc. In present study all the tracts were observed to be either strongly or intensely positive for SD as well as G 3 Pd. Similar findings on their distribution in the nrrvous tissues of higher vertebrates are conflicting. In developing rat central nervous system, Laatsch (1962) pointed out the G 3 PD involvement in the biosynthesis of membrane phospholipids and activity was shown to increase markedly in level concurrent with the myelination. Oligodendrocystes are confirmed to be the main cell types expressing G3 PD in brain (Cammer and Zimmerman, 1983).. In adult pig brain, it was again observered that G 3 PD was more active in oligodendrocytes than in other brain fractions (Montz et al., 1985). Manocha and Stantha (1969) described predominant SD activity in dentritic and axonic branches in certain regions of b rain of squirrel monkey (Saimiri sciureus).

Complete absence of SD activity had been noted in the axons ot rat cerebellum (Tewari and Bourne, 1962). Friede (1966) also observed complete absence of SD in axons. However, it was reported in glomerular area of frog olfactory bulb (Teweri and Sood, 1974), intense SD activity in the glomeruli of mice, rats, rabbits, guinea pig and few other mammals (Shimizu and Morikawa, 1957) including in the synaptic ureas (Kummamoto et al., 1965).

B. bendelisis is surface dweller and sight feeder implying that visual sensens are of prime importance therefore, the brain areas controlling the photo functions and allied activities reflect the high degree of energy turnover and necessity of myelin syntheis and its maintenance. Various mesencephalic tracts also appear to be actively involved in the transmission of sensory and motor impuses. High concentration of these two dehydrogenases observed in the present study (possibly other dehydrogenases also) prove the contention and indicate the corresponding involvement in visual functions.

High degree of these enzymes, particularly of G 3 PD, in fibre connections, suggest their role in biosynthesis and maintenance of myelin (Laatsch, 1962; Montz et al., 1985). Friede (1966) stated, in higher vertebrates, that the patterns of dehydrogenases distribution, SD in particular, in brain areas are directly indicative of degree of capilarization, rate of tissue respiration and hence energy metabolism, distribution and orientation of mitochondrial population. It appears to be plausible as far as fish brain is concerned as evident from the present study. It may also be concluded that glucose is the main energy liberating fuel in the fish brain and it metabolises through E-M pathway anaerobically in cytoplasm and then aerobically in mitochondria indicative of rich population of mitochondria in axoplasm. There are also possibilities of existance of hexose monophosphate shunt patway to supplement the main pathway during exigencies, if any. However, it needs further confirmation.

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ROZMIESZCZENIE I AKTYWNOŚĆ DEHYDROGENAZ W ŚRODMÓZGOWIU KARPIA *BARILIUS BENDELISIS* (HAMILTON)

STRESZCZENIE

W niniejszej pracy zajęto się lokalizacją i określeniem aktywności dwóch enzymów oksydatywnych, tj. dehydrogenazy fosforanowej glicerolu3) G3 PD EC.1.1.1.8) i dehydrogenazy bursztynianowej (SD EC 1.3.99,1) w ciałach jądrzastych i różnych układach włókien śródmózgowia *Barilius bendelisis*. Obszary jądrzaste śródmózgowia charakteryzowała słaba lub średnia aktywność obu oznaczanych enzymów. Wysoką aktywność obu dehydrogenaz zaobserwowano we włóknistej warstwie siatkówkowej pokrywy ocznej (STOR), włóknach warstwy centralnej (STAC) i szeregu innych układach włókien. Jednocześnie podobny był schemat aktywności obu enzymów. Bardzo wysoka lub wysoka atywność G 3 PD i SD notowana była także w układach włókien łączących tak części śródmózgowia jak i śródmózgowie z innymi częściami mózgu.

Stwierdzono, że G 3 PD zdaje się wspomagać biosyntezę mieliny i jej utrzymywanie w mózgu *B. bendelisis*. Glukoza zdaje się być głównym surowcem energetycznym, metabolizowanym najpierw beztlenowo w cytoplaźmie a następnie z udziałem tlenu w mitochondriach. W przypadku niedoborów energetycznych uruchamiany jest, prawdopodobnie. boczny cykl pentozowy, wspomagający cykl główny pozyskiwania energii.

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