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Research Article

HISTOENZYMIC DISTRIBUTION IN DIFFERENT PARTS OF BRAIN IN BUFFALO FOETUS

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ABSTRACT

The present study was conducted on brain of 12 buffalo foetuses obtained from the abattoir. The fresh tissue samples from different parts of fore, mid and hind brain were collected immediately after slaughter and subjected to cryostat sectioning at -20°C. The sections of 10-12 µm thickness were obtained and incubated in different substrates for demonstration of phosphatases, oxidoreductases and estrases. The study revealed a variable activity of phosphatases (AKPase, G-6-Pase), oxidoreductases (SDH, LDH, NADH AND NADPH) and estrases (AchEase) in different parts of brain in buffalo foetus. Strong AKPase activity was observed in the blood vessels, meninges and choroid plexus of lateral and fourth ventricle of the brains in all age groups, whereas the activity of SDH and LDH varied from moderate to strong. The intense activity of NADH and NADPH was observed in the choroid plexus of lateral and fourth ventricle, but AchEase activity varied from mild to moderate. It may be interpreted from the present study that the localization of different enzymes is variable due to the proliferation of various cellular components in the developing brain of buffalo.

Key words: Buffalo foetus, brain, histoenzymology

The enzyme histochemistry of brain is very important to determine the neurological disorders in foetuses from maternal exposure to xenobiotics (Chieco *et al.,* 1988). This method preserves the structural architecture and provides topological information on the distribution of various enzymes in different parts of brain (Kugler, 1988). Some studies have been reported in the distribution of enzymes in brain of pig foetus (Niespodziewanski, 1964), goat foetus (Lucy, 2005) and mouse brain (Tam and Kwong, 1987), but scanty information is available on the distribution of enzymes in the buffalo. Therefore, the present study was conducted to correlate the functional activity of brain during different parts of brain in foetus.

MATERIALS AND METHODS

The present study was conducted on brain of 12 buffalo foetuses obtained from abattoir. The foetal body length was measured as curved line in centimetre with the help of inelastic thread along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and designated as crown rump length (Edward, 1965). The approximate age of the foetuses was calculated by using the formula given by Soliman (1975) and foetuses were divided into three groups based on their Curved Crown Rump Length (CVRL) i.e. Group I (between 0 – 20 cm), Group II (> 20 to 40 cm) and Group III (above 40 cm).

The fresh tissue samples from different parts of fore, mid and hind brain were collected immediately after slaughter and subjected to cryostat sectioning at -20°C. The sections of 10-12 µm thickness were cut and incubated for demonstration of various enzymes viz; alkaline phosphatase (AKPase), G-6-Phosphatase (G-6-Pase) by coupling azodye method (Barka and Anderson, 1963), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), Reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase) and Reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) by nitro BT method (Pearse, 1972) and acetyl cholinesterase (AchE) by Butcher's method (Chayen *et al.,* 1969). The positive and negative controls were carried out wherever possible.

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RESULTS AND DISCUSSION

The localization of phosphatases, oxidoreductases and esterasesin different parts of the buffalo foetus brain are summarized in table 1.

Phosphatases

The blood vessels in the cortical zone of cerebrum showed a strong reaction for AKPase in buffalo foetus at different age groups. A fine granular AKPase activity was observed in the meninges and subarachanoid space and arachanoid meninges (Fig. 1). The choroidal epithelium of fourth and lateral ventricle showed moderate to strong AKPase reaction in its apical part (Fig. 2). In general, strong AKPase activity was observed in the blood vessels, meninges and choroid plexus of lateral and fourth ventricle of the brains in all age groups. Similar observations have been reported in pig foetus (Niespodziewanski, 1964), goat foetus (Lucy, 2005) and mouse brain (Tam and Kwong, 1987). The strong AKPase activity in the choroid plexus, blood vessels and meninges is suggestive of better ionic movement across the wall which is required for the secretory activity. Alkaline phosphatase activity may be related to an enhanced metabolism occurring during the formation of cranial nerve efferent nuclei and the establishment of neuronal circuits in the foetal brain (Tam and Kwong, 1987). Findings of Yoshioka *et al.* (1988) suggested that AKPase may play an important role in the membrane activity of epithelial cells differentiating between the primitive epithelial cells of the ventricular roof and the mature choroidal epithelial cells and possible relationship between the cerebrospinal fluid and the nervous tissues through the ependymal linings. A very weak activity of glucose 6 phosphatase was observed in different parts of foetal buffalo brain.

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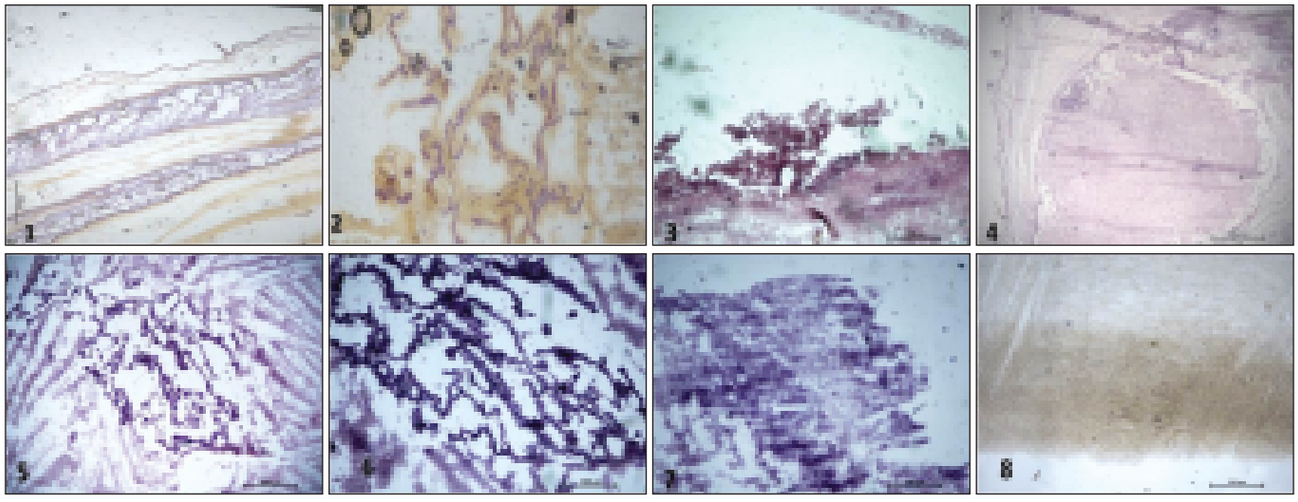

Fig. 1: 80 cm CVRL (254 days) buffalo foetus showing strong alkaline phosphatase activity in the arachnoid and subarachnoid layers.Azodye method X 400; **Fig. 2:** 31.3 cm CVRL (144 days) buffalo foetus showing alkaline phosphatase activity in choroidal epithelium of lateral ventricle. Azodye method X 200; **Fig. 3:** 31.3 cm CVRL (144 days) buffalo foetus showing moderate to strong SDH activity in the choroid plexus of lateral ventricle. Nitro BT method X 40; **Fig. 4:** 6.8 cm CVRL (59 days) buffalo foetus showing mild to moderate LDH activity in choroid plexus of fourth ventricle and mild activity in cerebellum. Nitro BT method X 40; **Fig. 5:** 80 cm CVRL (254 days) buffalo foetus showing moderate to strong NADPH activity in the choroid plexus of lateral ventricle.Nitro BT method X 40; **Fig. 6:** 80 cm CVRL (254 days) buffalo foetus showing moderate to strong activity of NADH in choroid plexus of lateral ventricle. Nitro BT method X 40; **Fig. 7:** 80 cm CVRL (254 days) buffalo foetus showing moderate activity of NADH in cerebral cortex and weak to mild activity in lateral ventricle. Nitro BT method X 40; **Fig. 8:** 31.3 cm CVRL (144 days) buffalo foetus showing moderate activity of acetyl cholinesterase in gray matter and weak to mild activity in white matter of cerebral cortex. Thiocholine method X 40.

Oxidoreductases

Fine granular SDH activity of moderate to strong intensity was observed in choroid plexus, blood vessels and meninges of brain (Fig. 3). This may be correlated with the secretory activity of choroid plexus in foetal life. Different layers of cerebral cortex showed various intensity of SDH. It was found to be moderate in the ventricular and subventricular zone, weak to mild in intermediate zone and mild to moderate in cortical plate. The LDH activity was found to be mild to moderate in different compartments of the brain. The activity was reported to be mild to moderate in the choroid plexus of fourth and ventricle, and weak to mild in cerebrum and cerebellum (Fig. 4). Rubleva and Solov'eva (1983) explained that the LDH-associated activity was differentiated from the activity of succinate dehydrogenase, which oxidizes the endogenous tissue substrate. They further added that LDH was localized in mitochondria and hyaloplasm, while the activity in the plasma membranes and lamellar structures is largely related to succinate dehydrogenase functioning.

The intense activity of NADH and NADPH was observed in the choroid plexus of lateral and fourth ventricle, whereas weak reaction was observed in ependymal lining of lateral and fourth ventricle of buffalo foetus (Figs 5 and 6). The ventricular zone of cerebrum showed moderate activity at 80 cm CVRL (Fig. 7). Enzyme pattern reflects general gradation of oxidative energy metabolism (Friede and Fleming, 1962). As NADH is a coenzyme of dehydrogenase and its activity may be an indicator of higher metabolic rate required for cellular differentiation and cellular proliferation.

Esterases

A mild to moderate AchE positive reaction was observed in all the parts of the brain. In the prosencephalon, the activity was more in the gray matter than the white matter (Fig. 8). The reaction was more towards the marginal zone than the other layers of cerebral cortex. Similar observation was made by Chernyshevska (1991) in human and mammalian brain, whereas reverse pattern was noticed in different parts of the brainstem in buffalo foetus. It was noticed that the activity was less in group I and II as compared to group III. Similarly Adejumo (1992) found the low AchE activity in foetal goat brain which increased between 4-14 weeks of gestation. Present findings are supported by Adejumo and Egbunike *(*2004), who reported increased AchE in the cerebellum of day old large white pigs. This may be attributed to the presence of more neurons, neuroglial cells and nerve fibers in the developing brain.

AchE positive nerve fibers could be demonstrated in the intermediate zone and cortical plate of cerebral cortex and extra granular layer of cerebellum. Similarly, Chernyshevska (1991) observed highest AchE activity in intermediate and plexiform layer of cerebral cortex of human foetii.

It may be interpreted from the present study that the localization of different enzymes is variable due to the proliferation of various cellular components in the developing brain of buffalo.

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