SECTION - I

STUDIES ON BRAIN SPECIFIC ACIDIC PROTEINS

1. Changes in brain specific S-100 protein in the developing albino rats on injecting sublethal dose of methyl parathion
INTRODUCTION

The role of macromolecules, especially the proteins has been well recognised in the nervous system (Sharma, 1980). Vertebrate nervous system is known to contain a high proportion of acidic proteins (Moore and McGrerger, 1965). The presence of acidic proteins in the brain is intimately connected with cell differentiation and functional specificity of the cells (Sharma, 1980). S-100, primarily a glial protein is the first that was purified from beef and rabbit brain (Moore, 1965). It has been found to be specially related to learning processes (Hyden and Lange, 1970).

S-100 is a brain specific acidic protein. It exists predominantly in soluble form, and occurs in the range of 0.1-0.2% (Moore, 1972, 1975) of the total brain soluble protein. This protein is named as S-100 because of its solubility in 100% saturated ammonium sulphate at pH 7.0. It is a typical globular simple protein with fast migration characteristics in gel electrophoresis (Moore, 1965; Cicero and Moore, 1970). S-100 protein is highly acidic in nature and about 30% of its amino acids are glutamic and aspartic acids (Uyemura et al., 1971; Stewart, 1972). It is heat stable in presence of $1\times10^{-3}$M EDTA, 2-mercaptoethanol and $\text{CaCl}_2$ (Sharma, 1980).
S-100 protein is found to be distributed in all parts of the nervous system both peripheral and central (Moore and Perez, 1968; Zanini and Angcletti, 1968). Its concentration is higher in cerebral white matter than in cerebral cortex. In human brain, its highest concentration was observed in white matter (occipital, frontal, parietal, cerebellar) and the lowest in gray matter (Sharma, 1980). In rabbit brain it is generally found in higher concentrations in cerebellum, within the cerebellum, molecular layer has highest concentration followed by the granular layer and white matter.

Appearance and accumulation of S-100 protein postnatally in the developing rat brain has been studied (Herschmann et al., 1971, Stewart and Urban, 1972; Cicero et al., 1972). Its presence detected in the rat brain on the fifth day after birth. It begins to accumulate in the forebrain, brain stem and cerebellum between 7th and 14th day after birth (Cicero et al., 1972). Its concentration increases sharply in all the regions of brain except forebrain. Forebrain accumulated it from day 14 to 21. Appearance and accumulation of S-100 protein in the brain stem and cerebrum has been observed postnatally (Herschmann et al., 1971), at the end of proliferation period.

Immunological technique shows that S-100 protein is located primarily in the cytoplasm of certain glial cells,
where it is synthesized (Moore, 1975). Its presence is reported in neuronal nuclei (McEwen and Hyden, 1966; Hansson et al., 1975), neuronal perikarya (Hyden and Lange, 1970) synaptosomes (Donato, 1976 and Donato et al., 1975) and in postsynaptic densities (Haglid et al., 1974). The synthesis of S-100 protein has been detected in glial cells but has a site of action on or in neurons (Shashova et al., 1984). Neuronal S-100 protein migrates from soma to terminal of the hypoglossal vagus and glassopharyngeal nerves of rabbits (Miani et al., 1972).

S-100 protein has also been found to be associated with the cellular structures (Rusca et al., 1972; Haglid and Stavrow, 1973). This membrane bound fraction represents 20-25% of the total S-100 protein present in soluble fraction. It is now documented that S-100 protein fraction represents a group of proteins (Sharma and Talwar, 1972). Micheli et al. (1974) have indicated the presence of three forms of S-100; free S-100, in the soluble protein fraction; liable bound S-100 present in the deoxy ribonucleoproteins and stable bound S-100, present in the residual fraction. The nuclear S-100 is a small fraction constituting about 0.55% of the total cytosol.

The biological functions of S-100 protein are not precisely known, though it has been shown to be an important
functional protein in the nervous system (Talwar and Iqbal, 1972; Sharma, 1974). It is known to have high binding affinity for calcium (Moore, 1972) and a close structural homology with troponin-C (Isobe and Okuyama, 1981). This suggests a possible role of S-100 protein in controlling some aspects of calcium availability in the CNS. S-100 protein binds with a high affinity to synaptic membranes in the presence of calcium (Donata, 1976) and reportedly alters the calcium transport properties of synaptosomal membranes (Gallo et al., 1980) and potassium and calcium permeability of liposomes (Calissano et al., 1974).

The patterns of accumulation and appearance of S-100 protein in the developing nervous system coincide with the maturation of electrophysiological activity in a variety of animal species (Herschman et al., 1971; Stewart and Urban, 1972; Cicero et al., 1972; Cicero and Provine, 1972). Experimental evidence indicates a correlation between the neurophysiological functions like learning and memory and the content of S-100 protein in the brain of mammals (Hyden and Lange, 1970). They found an increase in the content of S-100 protein in pyramidal nerve cells of the hippocampus during learning in rats. The increase in the content of S-100 protein during training of the animal have been found to coincide with the rise in calcium in the CA3 region of the hippocampus (Haljamae and Lange, 1972).
Hyden and Lange (1976) reported that a change in the level of S-100 protein disturbs the memory and learning process in the hippocampus. Karpiak et al. (1976) found that an injection of anti S-100 serum (by the intraventricular route) inhibited the performance of rats in a maze-learning task. Calcium ions have been shown to induce conformational changes in S-100 protein (Moore, 1972).

A number of spontaneous and experimentally induced brain tumors and clonal cultures of rat and human glial cells, have been reported to accumulate S-100 protein (Pfeiffer et al., 1970, 1972; Zomzely-Neurath et al., 1973; Labourdette and Marks, 1975). Hyden and Ronnback (1979) reported that alterations occur in the content of S-100 protein during applied external sensory stimuli in rats.

Since organophosphates are nerve poisons, it is likely that they affect acidic proteins of the nerves system. Working with the amphibian brain during critical stage of CNS development Naiyara Yasmeen (1989) found a decline in S-100 level on administration of methyl parathion. However, information is entirely lacking on S-100 proteins during insecticide toxicity in a developing mammalian brain. Keeping this in view, and the importance of S-100 protein in the functioning of developing brain, the present study was proposed. S-100 proteins were resolved in the discrete areas
of developing mammalian brain during methyl parathion induced toxicosis.

MATERIALS AND METHODS

Developing albino rat pups (2 and 7-day-old) were injected with sublethal doses of methyl parathion. They were decapitated 48hrs after insecticide administration and discrete areas of brain were separated for analysis at 0°C as described in general materials and methods.

S-100 Protein

S-100 protein was extracted following the procedure of Watterson et al. (1976) and was resolved by polyacrylamide gel electrophoresis. One gram of brain tissue was homogenized in 2-volumes of 0.1M sodium acetate, 0.001M 2-mercaptoethanol and 0.001M EDTA, pH 7.2 and centrifuged for one hour at 10,000rpm at 4°C. The supernatant fluid was decanted and saved. The pellet was homogenized in an equal volume of buffer, centrifuged again, and the resulting supernatant fluid added to the original supernatant fraction. The pellet was discarded.

S-100 protein was extracted by adding solid ammonium sulphate to the supernatant fraction to bring the solution to 50% saturation. The resulting solution was adjusted to pH 7.0 with 1N NH₄OH and centrifuged for 30min. at 10,000rpm.
The supernatant fluid was decanted and adjusted to pH 4.0 with 1N sulphuric acid in 50% ammonium sulphate. The mixture was stirred well and centrifuged at 10,000 rpm for 30 min. and the supernatant fluid discarded. The pellet was resuspended in an equal volume of buffer and subjected to discontinuous polyacrylamide gel electrophoresis (12.5%).

Discontinuous polyacrylamide gel electrophoresis was performed using tube gels consisting of 12.5% (w/v) of acrylamide, 0.03% (w/v) bisacrylamide, 4.5% (v/v) Tris, 0.06M HCl, 0.025M (w/v) N,N,N',N'-tetramethylethylenediamine and 0.75% (w/v) ammonium persulphate. The upper reservoir contained 0.05M Tris base, 1mM EDTA, 0.4% (w/v) glycine and 0.07% (v/v) 2-mercaptoethanol. The lower reservoir contained 0.1M Tris base/0.05M HCl. Sample aliquots were mixed with an equal volume of 0.01% (w/v) bromophenol blue in 50% (v/v) glycerol and applied to the gels. Electrophoresis was performed at 150 volts for 5hrs. Gels were stained with 0.25% (w/v) Comassie brilliant blue R250 in 50% (v/v) methanol and 10% (v/v) acetic acid for 1 hour at 37°C and destained for 2 hours in 7.5% (v/v) acetic acid and 5% (v/v) methanol at 37°C.

The stained gels were passed under a gel densitometer (Biochem Instruments India). The readings were recorded for every mm of the gel distance moved and the graphs were prepared.
RESULTS

Figs. (1-4) clearly depicts the electrophoretic pattern of brain specific acidic protein viz. S-100 protein in discrete areas of CNS of control and methyl parathion treated developing rats. S-100 protein could be resolved both in the 2nd and 7th day old developing albino rat pups. From the figures, it is clear that through polyacrylamide gel electrophoresis S-100 protein could be resolved as a single defined band. It is also evident from the figs. (1-4) that S-100 protein showed the fastest mobility and hence tracks with the dye. In general, methyl parathion exposure has decreased significantly the level of S-100 protein in the cortex and medulla.

In 2nd day-old rat pups the level of S-100 protein decreased significantly in cortex (-40.0%) and brain stem (-39.9%) on methyl parathion exposure (Figs. 1-2). Even in 7-day-old rat pups the S-100 protein decreased significantly in cortex (-33.33%) and brain stem (-18.2%). In 7-day-old animals the S-100 level was comparatively more indicating that S-100 level increased during post natal development (Figs. 3-4).

DISCUSSION

Figs. (1-4) depict the pattern of S-100 protein in the cerebral cortex and brain stem of control and methyl
parathion exposed developing rats. In the present study S-100 protein showed the fastest mobility (Figs.1-4). It is clear from the present study that S-100 protein decreased considerably during methyl parathion induced toxicity.

Brain specific function has been approached by attempting to detect and purify proteins unique to the nervous system (Moore and McGreger, 1965). The studies on brain specific proteins are important because it appears probable that in pathological conditions leakage of mobile water soluble intracellular proteins can occur more readily than that of structure bound proteins (Marcvan Sande, 1972; Kandel and Schwartz, 1985). S-100 protein is largely confined to the nervous system and is found in a variety of vertebrates with serological similarity among various species (Sharma, 1980). S-100 protein is known to have high affinity for calcium and a close structural homology with tropanin 'c', therefore may have a possible role in controlling some aspects of calcium availability in the CNS (Shashoua et al., 1984). Experimental evidence indicates a correlation between the neurophysiological functions like learning and memory and the content of S-100 protein in the brain of mammals (Sharma, 1980). In view of the facts presented above the significant decrease observed in S-100 protein content of the developing CNS in the present study, can be taken to be an index of suppression of learning and
memory processes during toxicity induced by methyl parathion. This decrease in S-100 content of the brain is therefore indicative of decreased neuronal efficiency during methyl parathion induced toxicity. It is possible that the decrease in S-100 level in the brain during methyl parathion imposed toxicity causes disturbance in the calcium availability in the CNS thereby disturbing the molecular events like elicitation and transmission of nerve impulses. It is likely that the impaired calcium availability affects profoundly the transmitter release during methyl parathion exposure.
Plate 2: Discontinuous polyacrylamide gel electrophoresis of S-100 protein in discrete areas of brain of 2-day-old rat pups

Photographs of stained gels representing the S-100 band (S) in cortex and brain stem areas of control and methyl parathion treated rat pups.

A. Control Cortex  
B. MPT Cortex  
C. Control brain stem  
D. MPT brain stem

Plate 3: Discontinuous polyacrylamide gel electrophoresis of S-100 protein in discrete areas of brain of 7-day-old rat pups

Photographs of stained gels representing the S-100 band (S) in cortex and brain stem areas of control and methyl parathion treated rat pups.

A. Control Cortex  
B. MPT Cortex  
C. Control brain stem  
D. MPT brain stem
Fig. 1: Discontinuous polyacrylamide, gel electrophoresis of S-100 protein, in the cerebral cortex of 2-day-old rat pups.

Lines with closed circles represent the electrophoretic pattern of S-100 protein in the control cerebral cortex.

Lines with open circles represent the electrophoretic pattern of S-100 protein in the methyl parathion treated cerebral cortex.

Representation of gel is semidiagramatic.
FIGURE 1

Absorbancy

Distance in mm

C

MPT
Fig. 2: Discontinuous polyacrylamide gel electrophoresis of S-100 protein, in the brain stem of 2-day-old rat pups.

Lines with closed circles represent the electrophoretic pattern of S-100 protein in the control brain stem.

Lines with open circles represent the electrophoretic pattern of S-100 protein in the methyl parathion treated brain stem.

Representation of gel is semidiagramatic.
Fig. 3: Discontinuous polyacrylamide, gel electrophoresis of S-100 protein, in the cerebral cortex of 7-day-old rat pups.

Lines with closed circles represent the electrophoretic pattern of S-100 protein in the control cerebral cortex.

Lines with open circles represent the electrophoretic pattern of S-100 protein in the methyl parathion treated cerebral cortex.

Representation of gel is semidiagramatic.
FIGURE 3

Absorbancy

Distance in mm

C

MPT

50 60 70 80 90
Fig. 4: Discontinuous polyacrylamide, gel electrophoresis of S-100 protein, in the brain stem of 7-day-old rat pups.

Lines with closed circles represent the electrophoretic pattern of S-100 protein in the control brain stem.

Lines with open circles represent the electrophoretic pattern of S-100 protein in the methyl parathion treated brain stem.

Representation of gel is semidiagramatic.