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CHANGES IN PHOSPHOLIPIDS AND ACETYLCHOLINESTERASE DURING EARLY PHASE OF INJURY TO SPINAL CORD – AN EXPERIMENTAL STUDY IN RATS

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Abstract : Injury to spinal cord was produced in rats by the clip compression technique by placing the aneurysm clip extradurally for 30 seconds. The traumatised spinal segment and the adjoining upper segment were used for biochemical estimations. Motor function of the injured rats was evaluated using the inclined plane. Phospholipid phosphorus values were significantly decreased in the injured spinal segment at 24 hrs. AchE activity was also decreased in the traumatised segment one week after injury. Dexamethasone and verapamil reversed the changes in AchE activity at the end of one week. At the one week assessment period, aneurysm clipped rats showed a decrease in the maximum angle in the inclined plane. Dexamethasone and verapamil treated rats showed improvement in the neurologic function, neurologic recovery was better in the dexamethasone treated group.

Key words : spinal injury phospholipids verapamil dexamethasone acetylcholinesterase

INTRODUCTION

Injury to the spinal cord can be the result of mechanical damage caused by impact force or persistent compression caused by space occupying lesions or bone fragments. The acute phase of spinal cord injury is associated with mechanical damage leading to alterations in blood supply (1, 2) and biochemical parameters. Biochemical changes such as lactate accumulation (3) alterations in the biogenic amine levels (4) and generation of free radicals (5) have been reported in various studies. Lipid hydrolysis with subsequent eicosanoid production is a biochemical event during the early phase of the spinal cord injury (6). The increase in intracellular Ca^2 (7) is known to be one of the factors that may initiate the enzymatic and lipid changes. This study was designed to observe changes in phospholipids and acetylcholinesterase, in spinal cord tissue following compression injury. The influence of dexamethasone and verapamil were observed on the changes in acetylcholinesterase during one week following injury. Dexamethasone is reported to improve functional outcome in animals studies (8, 9). Verapamil is known to influence the movement of calcium from extracellular to intracellular sites.

METHODS

Adult Wistar rats weighing 180-200 g of either sex were used for the study. Rats were anaesthetised with pentobarbitone sodium (50 mg/kg). Injury was produced by the extradural clip compression technique (10). A laminectomy was performed from C7 to T2. The technique consisted of placing a modified Kerr-Lougheed aneurysm clip around the cord

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extradurally. The arms of the clip were 1 mm wide, 0.5 mm thick and 8 mm long were curved to facilitate extradural placement around the cord. Compression injury was produced at the level of T1 by placing the clip extradurally for 30 seconds. The traumatised segment (SI) and adjoining upper segment of 1 mm length (S2) were removed for biochemical estimations. Sham operated controls had laminectomy done from C7 to T2 and the corresponding spinal segments were removed after one week from these rats. Immediately after surgery all rats received gentamycin 0.2 mg/100 g body weight (IM). The urinary bladders of all operated animals were manually compressed every 12 hours for one week following surgery. Water and food were made available *ad libitum*.

Dexamethasone and verapamil were administered daily for 7 days following injury. Dexamethasone was given in doses of 0.5 mg/kg on days one and 2, 0.25 mg/kg on days 3 and 4; 0.125 mg on days 5, 6 and 7. Verapamil was administered in doses of 0.2 mg/kg on days 1, 2 and 3; 0.4 mg/kg on days 4 and 5; 0.8 mg/kg on days 6 and 7. Control injured rats received saline.

The animals were evaluated for motor function for one week. Motor function was carried out on an inclined plane once in 2 days for 7 days. A blind testing method was used whereby the observer was not aware whether a given animal was control or a treated animal. The inclined plane consisted of a adjustable plank covered by a rubber mat constructed after the apparatus described by Rivlin and Taylor (11). The angle of the plane was increased from 0° until the rat could not maintain its position for 5 sec. Rats were placed on the inclined plane and the maximum angle at which the rat could maintain its position for atleast 5 sec without falling was measured.

Biochemical studies : Acetylcholinesterase (AchE) and phospholipids were estimated in spinal cord segments. Phospholipids were estimated 24 hrs after injury without any drug administration. AchE was estimated in spinal segments from sham controls and drug treated rats one week after injury.

AchE : Spinal segments were cleaned of blood, weighed and homogenates were used for enzyme assay. AchE was assayed by the method of Ellman et al (12) using acetylthiocholine as substrate. Butyl cholinesterase (BuChE) activity was also estimated using butyl thiocholine as substrate and in the presence of BW 284C51, a specific inhibitor of AchE to ascertain any activity due to BuChE. The specific activity of AchE was found to be inhibited (98%) when BW 284C51 was used as an enzyme inhibitor. This suggests that most of the activity is due to AchE. The specific activity of the enzyme was expressed as units of enzyme activity per mg protein. The protein was determined by the method of Lowry et al (13).

Phospholipids : The concentration of total and individual phospholipid in spinal cord segments were determined in chloroform - methanol extracts. Total lipid extracts were prepared by the method of Folch et al (14) by homogenizing the spinal cord tissues in 5 ml chloroform - methanol - 12 MHcl. Phospholipids were isolated by two dimensional thin layer chromatography (14). Separated phospholipids were visualised by exposure to iodine vapors and iodine positive spots were quantitated after acid digestion (15).

Statistical significance was tested by Students' t-test and a level of $P < 0.05$ was considered as significant. Drug treated groups were compared with saline treated injured rats.

RESULTS

The concentration of phospholipid is expressed as a function of wet weight of tissue. Total phospholipid phosphorus showed a significant decrease in the injured spinal cord segment at 24 hrs. Both phosphatidyl choline and phosphatidyl ethanolamine was found to be decreased in the injured segment (Table I).

AchE activity was decreased in the traumatised segment I after one week in the

TABLE 1 : Changes in phospholipid content in spinal cord injured rats.

Group	*Total phospholipids*	*Phosphatidyl ethanolamine*	*Phosphatidyl choline*
Controls	62.02±6.13 ***	16.56±0.52 **	12.43±1.24 *
Injured	40.17±2.76	09.91±1.50	07.82±1.00

P values less than *0.05 **0.01 ***0.02

Phospholipid values are expressed as nmol/mg wet weight. Number of rats in each group is 4.

injured rats. No significant change in the activity was observed in segment II. Dexamethasone in the given doses were found to reverse the decrease in AchE activity in the S1 segment of the injured rats (Fig. 1).

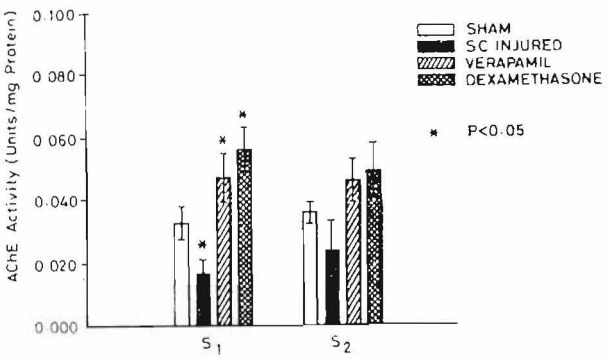

Fig. 1 : Changes in AchE activity in the spinal cord of rats S1, injured segment, S2 uninjured adjacent segment. Data are mean SEM of 4 rats in each group. Injured group is compared with sham controls, drug treated groups with the injured group.

Studies on neurologic function showed that sham operated rats could maintain a maximum angle of 76.77±1.01 in the inclined plane (Fig. 2). At one week assessment period, aneurysm clipped rats showed a decrease in the maximum angle in the inclined plane. The dexamethasone treated rats could maintain themselves at a higher angle than the injured rats at the end of one week. Verapamil treated rats also showed an improvement in the neurologic function. Neurologic recovery was better in the dexamethasone treated group (P<0.001).

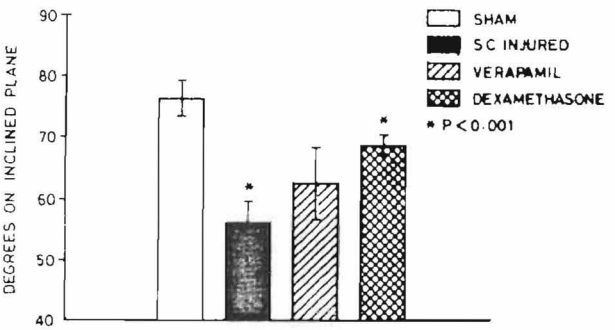

Fig. 2 : Motor function as observed on the inclined plane during one week after injury. Data are mean ± SEM of 4 rats in each group. P values are indicated *less than 0.001. Injured group is compared with sham controls. Drug treated groups are compared with the injured group.

DISCUSSION

Results of this study show a decrease in phospholipid content in the injured tissue. Lipid hydrolysis and liberation of products of hydrolysis such as phospholipids and diacylglycerol are known to perturb the integrity of membranes (16). Prostaglandin levels are reported to be increased at the site of neuronal injury (17). Lipid peroxidation and generation of free radicals are some of the other biochemical events detected during the early phase of spinal cord injury (5, 6).

Change in membrane phospholipids may alter the activity of the enzymes which are functionally related to neuronal membrane. In the present study the activity of enzyme AchE is found to be decreased in the injured segment. Similar results have been observed in primates following spinal cord injury induced by weight drop (18). These changes are reversed by test drugs dexamethasone and verapamil. In the study on primates beneficial effects have been observed by these drugs on the various biochemical parameters such as AchE activity, NaKATPase and lysosomal enzymes during early phase of contussion injury. High dose of dexamethasone was reported to be effective in spinal cord compression (8). Weidefeld et al (19) has reported that dexamethasone is effective in lowering prostaglandin production in the brain.

The reported cerebroprotective mechanisms of methyl prednisolone include inhibition of post traumatic lipid peroxidation (20, 21) reversal of intracellular calcium accumulation (22) and prevention of neurofilament degradation (23).

Normal nerve cells maintain a remarkable calcium iongradient between intracellular and extracellular compartments. Ischaemic or traumatic insults to the neurones can alter this balance (24). These changes are attributed to the increase in cell membrane permeability and consequent influx of Ca^{2+}. Desphpande et al (25) have demonstrated that calcium accumulates before cell necrosis occurs implying that change in calcium homeostasis is an early event in neuronal injury. Accumulation of calcium in the intracellular space is considered as a factor activating phospholipase A and C, which will attack membrane phospholipids. Verapamil treatment for one week partially reversed the changes in enzyme activity of injured segment and produced partial inprovement in neuronal function. Verapamil may produce these effects by reducing the toxic effects of calcium accumulation and further damage to membranes.

In summary, this study highlights the biochemical changes observed during early phase of spinal cord injury induced by compression. The test drugs dexamethasone and verapamil reversed the change in acetylcholinesterase activity and improved neurologic function partially.

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