DISCUSSION

DRUG EFFECTS ON SPINAL INJURY

Primate injury model: A modified Allen's weight drop method was used, to induce injury. The site of injury was at D7 in all experiments. An impounder injury of 200 gm-cm force produced transient paraplegia in all animals. Preliminary studies show that the force of impact and the intensity of trauma are correlated in this primate model. Use of a primate model for this study would have more relevance to the clinical situation. There is considerable amount of variability in the nature and the symmetry of the impact on the spinal cord. Animals which produced a constant injury, neurologically and pathologically were used in the experiments. With this type of injury, meningeal membranes were not cut; the meningeal integrity of the spinal cord was left intact. This aids in preventing the penetration of loose connective tissue and interstitial fluid. One of the most disadvantages of this method is that the most severe site of trauma in spinal injuries is on the ventral portion instead of the dorsal portion as seen in weight drop method. Another disadvantage of this method is that it requires a sizable dorsal laminectomy and durotomy.

Rat injury model: A compression injury was induced in rats at T1 level with a modified aneurysm clip. Rats were chosen
in this study because they are easily available and less expensive. A standardized degree of spinal cord injury was produced with this technique. One of the major disadvantage of this method is the unreliability of the clinical assessment of the degree of spinal cord injury. We have chosen animals with a uniform neurological deficit for the drug studies.

**Evaluation of drug effects:** Each drug evaluated were given 30 minutes after the injury, that is the minimum period a spinal cord injured patient can get treatment. The animals were followed for 1 week. In our study, a group of uninjured sham animals were also treated with the same drug regimen.

**Method of evaluation of motor function:** Evaluation of recovery following spinal cord trauma has largely been based on return of functions such as sensory perception, normal reflexes, voluntary motor movements and the ability to walk. A modified Tarlov scale was used to assess the motor function in a blind fashion in primates. The inclined plane method of clinical assessment was used in rats. The inclined method was very easy to use because of the rapidity and ease with which the maximum angle could be determined. The inclined plane method quantitatively assesses the animal's ability to prevent themselves from falling over, and assess the residual strength in the upper and lower limbs. In this study the pharmacotherapy was done in two different animal models and
two methods were used to evaluate the clinical assessment in blinded fashion.

HAEMODYNAMIC CHANGES IN SPINAL CORD INJURY

Blood pressure changes have long been known to occur in spinal cord injury, probably resulting from adrenal catecholamine release and ganglionic activation during injury. The mechanical disturbance of the spinal cord activates sympathetic discharges in the thoracic and lumbar ganglia, splanchnic nerves, and the adrenal glands. These systems in turn cause vasoconstriction and an elevation of the blood pressure (Mitchell, 1953; Bard, 1960). Alexander & Kerr (1964) had pointed out that the latency of the pressor response is too short to be explained by adrenal catecholamine release alone. The adreno-cardiac circulation time is said to average 15 seconds (Alexander & Kerr, 1964). Young, (1980) attributed the initial hypertensive peak to a neuronally and humorally mediated sympathetic change. It is well known that the mechanical disturbance of the spinal cord will cause massive neuronal activation of the somatic system. Sympathetic tracts in the lateral funiculi may be activated by contusion (Coote et al., 1966; Foreman, 1973; Illert, 1972). Acute hypertension could cause an increase in the amount of hemorrhage and edema surrounding the spinal cord lesion. Acute hypertension has been shown to increase edema and neuronal damage following experimental spinal cord injury (Rawe, 1978).
Dexamethasone given after 30 minutes injury, neither decreased nor increased blood pressure. Verapamil further decreased the post traumatic hypotension, indicating a significant microvascular action. The hypotension that accompanies treatment with verapamil may contribute to a decline in spinal cord blood flow in an animal that has lost autoregulation following trauma.

It has been reported earlier that naloxone improves post-traumatic hypotension, ischemia of the spinal cord and clinical recovery after experimental spinal cord injury (Faden, 1980; 1982; Flamm, 1982; Holaday, 1981). Wallace (1986), failed to show any improvement in post-traumatic hypotension and spinal cord ischemia with naloxone treatment. In the present study, we found a beneficial effect of naloxone in improving the post-traumatic hypotension. This hypertensive effect of naloxone is mediated by blockade of endorphin effects at opiate receptors in the CNS (Holaday, 1980).

**BIOCHEMICAL CHANGES**

**Acetylcholinesterase:** Acetylcholinesterase plays a key role in the normal functioning of the cholinergic system by rapidly hydrolysing the transmitter molecule, acetylcholine, in the synaptic cleft. In this study, anaesthesia does not seem to have any effect on AChE activity. Anesthetics, theoretically should decrease ACh and then raise AChE level. The changes
which observed after anaesthesia is due to vascular changes rather than due to deepening of anaesthesia. In our study, sodium pentobarbitone (30 mg/kg) could not produce any changes in AChE activity. Laminectomy alone did not produce any changes in acetylcholinesterase activity in primates. Laminectomy significantly reduced AChE level in rats. It has been reported that, laminectomy alone merely produces a perturbation in spinal cord biochemical homeostasis without causing any significant cell death. In rats, laminectomy might have caused vascular changes, which may be responsible for the decrease in AChE activity. In the CNS, AChE and many other neural proteins are synthesized in the perikaryon and transported to the nerve endings (Lubinska, 1964; Kasa, 1968). Axonal transport of AChE is fast and bidirectional (Partlow et al., 1972; Ranish, 1972). In this study, the specific activity of AChE was found to be significantly decreased in traumatized segments at 24 hours to 1 week post-injury. The specific activity of AChE was found to be inhibited (98%) when BW284C51 was used as an enzyme inhibitor. This suggests that, most of the activity is due to AChE. The decrease in AChE activity at 24 hours suggest that this may be due to neuronal changes. It has been demonstrated that the true AChE is primarily a neuronal enzyme (Giacobini, 1968) and it is located in the grey matter in the control spinal cord. It has been shown that grey matter becomes dead at four hours post-injury while white matter becomes maximally necrotic after 8-
24 hours (Balentine, 1978). Histochemical localization of AChE has indicated that, the synthesis and release of AChE are attributable to neurons and that AChE release was significantly reduced when microtubules and protein synthesis were disrupted with drugs (Oh, 1977). Our studies also show a decrease in the specific activity of AChE in the CSF of traumatized animals from 24 h-1 week. Earlier periods did not show any significant changes. AChE activity present in CSF is mostly of spinal cord and brain tissue origin and the changes in AChE activity may be due to changes in neuronal cells and may reflect neuronal AChE activity. Thus, either the synthesis or release could be impaired due to neuronal damage. Neuronal AChE is an enzyme important in the regulation of synaptic transmission, it is reasonable to assume that, changes in this enzyme might in some way be related to the impairment of the electrical activity in spinal injury. Both acetylcholinesterase and Na⁺, K⁺, ATP-ase were found to be decreased in cerebral ischemia (Mrsulja et al., 1978). Decreased activity of AChE is suggestive for the increased content and or non-controlled release of free acetylcholine (ACh). Inhibition of Na⁺, K⁺, ATP-ase indirectly releases ACh by increasing intracellular sodium levels (Meyer, 1981). Corticosteroids are widely used in spinal cord injury. Dexamethasone in the doses administered in this study was found to be beneficial in reversing AChE activity in injured segments. A membrane stabilizing effect of dexamethasone could
be expected. Impact trauma produces initial alterations on cell membranes. These alterations are: (a) specific alterations of membrane-bound enzymes (b) disturbance at the level of lipid-protein organization.

Acetylcholinesterase has long been regarded as a membrane-bound enzyme associated primarily with cholinergic systems. AChE seems to have non-cholinergic function also (Taylor, 1988). A reduction in intracellular concentration of AChE could bring about an increase in the concentration of ACh in the soluble cytoplasmic fraction and it could decrease ACh concentration in the synaptic cleft. Acetyl cholinesterase activity in spinal cord tissue is significantly higher than in CSF. However, since protein levels in spinal cord tissue are higher than CSF, the specific activity of AChE is higher in CSF. Dexamethasone has an effect on normal spinal cord, decreasing water content (DeLattre, 1989). In this study, when sham animals are treated with dexamethasone, specific activity of AChE was found to increased. The actual mechanism of this increase is difficult to interpret. Steroids are useful in preserving the cellular and vascular membrane integrity and stabilizes the white matter of the spinal cord in the presence of hemorrhagic necrosis (Black, 1971). Steroids have a protective effect on cell membranes and may help in preventing the disruption of intracellular lysosomes (Ashford, 1966). Steroids are also known to possess anti-inflammatory and
antiedemic properties.

Following spinal cord injury, extracellular calcium decreases and intracellular calcium increases (Stokes, 1983; Young, 1982). These increases in intracellular calcium are toxic to cells and may be a final common mechanism of cell death following injury to the CNS (Harris, 1982). The increased AChE activity with verapamil may be due to the attenuation of neuronal damage through vasodilatation, and prevention of excessive calcium influx into cytoplasmic and mitochondrial compartments. Verapamil, a calcium antagonist drug believed to act at the cell membrane of excitable tissues, in the doses given for one week, increased AChE activity in traumatized segments. Verapamil also increased AChE activity in the sham spinal cord segments. It has been shown that verapamil increases cellular AChE activity (Patterson, 1979). As verapamil alters AChE activity, the enzyme activity can be manipulated by calcium.

Dimethyl sulfoxide in the given doses, further decreased the specific activity of AChE in both segments suggesting the doses administered may be toxic to cells, which aggravates the neuronal death. The decrease in AChE activity with naloxone is surprising. Existing literature indicates that, morphine administration either decreases or increases AChE in nervous system (Ho, 1970; Datta, 1971; Mohankumar, 1985). In our study
the opiate antagonist naloxone decreased AChE activity.

The fall in enzyme activity with nifedipine may be due to the solvent, ethanol, which could disrupt the function of membrane-bound enzymes by acting directly on the enzymes themselves or by disrupting membrane lipid-protein interactions (Collins, 1984).

Dipyridamole the anti-platelet drug increases AChE activity in spinal segments. Dipyridamole maintains AMP in its cyclic form by both stimulating its enzymatic production and reducing its active degradation (Weiss, 1982). Cyclic AMP raises AChE activity in cultured neuroblastoma (Furmanski, 1971). The increase in AChE observed after dipyridamole may be due to its effect on cyclic AMP.

\( \text{Na}^+, \text{K}^+, \text{ATP-ase} \): The \( \text{Na}^+, \text{K}^+, \text{ATP-ase} \) activity has been found to be reduced in traumatized segments after one week. The scientific literature reveals considerable controversy concerning the role of \( \text{Na}^+, \text{K}^+, \text{ATP-ase} \) during primary and secondary ischemia. The role of membrane \( \text{Na}^+, \text{K}^+, \text{ATP-ase} \) in maintaining cellular ionic gradients and membrane potentials is well established (Skou, 1965). Enzymic activity of the membrane-bound \( \text{Na}^+, \text{K}^+, \text{ATP-ase} \) has been shown to depend on the structural integrity of the membrane (Sun, 1970, 1971; Skou, 1965). One of the precipitating pathways of irreversible
neuronal damage is thought to be an increase in intracellular calcium (Schanne, 1979; Farber, 1981; Siesjo, 1981; 1984). Demopoulos et al. (1980) reported that cell membrane damage in the central nervous system following cerebral ischemia and spinal cord injury may be induced by free radical reaction and lipid peroxidation. Synaptosomal Na⁺, K⁺, ATP-ase has been demonstrated to be a phospholipid-dependent membrane-bound enzyme (Wheeler, 1975), and is very susceptible to free radical reaction and lipid peroxidation (Sun, 1972). Clendenon et al., (1978) reported that the activity of this enzyme decreased as early as 5 minutes after spinal cord injury in dogs. Braughler and Hall (1982a, 1982b) demonstrated the relationship between the inhibition of activity of enzyme and lipid peroxidation following spinal cord injury. Na⁺, K⁺, ATP-ase is highly concentrated in CNS plasma membranes (especially synaptic membranes) and is a good marker for membrane integrity (Albers, 1965). Several interpretations are generally proposed to explain membrane enzymatic deficiency:— (a) changes in the conformational state of the protein per se, which cannot undergo allosteric modifications, (b) alterations of surrounding membrane phospholipids, resulting in changes of lipid-protein organization and c) inhibition of the enzyme by various endogenous degradation products (Averet et al., 1987). It has been shown that polyunsaturated fatty acids particularly arachidonic acid reduce the activity of Na⁺, K⁺, ATP-ase (Chan
et al., 1983b). Calcium ions inhibit $\text{Na}^+$, $\text{K}^+$, ATP-ase activity when exposed to intracellular enzyme sites (Schwartz et al., 1975). The present studies showed that, dexamethasone increases the $\text{Na}^+$, $\text{K}^+$, ATP-ase activity in the injured segments. Recently, it was demonstrated that steroids are taken up preferentially by the traumatized tissue in the brain (Kostron, 1983) and spinal cord (Braughler, 1983). The Golgi complex, lysosomes and multivesicular bodies which take up dexamethasone to a significant amount after the trauma (Kostron, 1983). Mechanical injury activates biochemical processes, that leads to structural and functional disruption in the cord. The primary sites of injury are the cellular and subcellular membranes of neurons, glia, and vascular endothelial cells. The membrane-bound enzymes of the CNS are dependant upon an intact membrane structure for their activity. The influence of lipid environment on the activity of $\text{Na}^+$, $\text{K}^+$, ATP-ase is well known (Hokin, 1972; Roelofsen, 1981). Exogenous addition of phospholipids failed to restore $\text{Na}^+$, $\text{K}^+$, ATP-ase activity in vitro, suggesting changes in membrane phospholipid do not affect $\text{Na}^+$, $\text{K}^+$, ATP-ase activity (Enseleit, 1981). The decrease in $\text{Na}^+$, $\text{K}^+$, ATP-ase is increased by dexamethasone: (a) by membrane stabilizing action, (b) antiedemic effect, (c) due to protective effect on energy metabolism, (d) suppression of free radicals induced lipid peroxidation, (e) inhibit phospholipases. Thus in this study, dexamethasone offered partial protection against injured
damage to Na\(^+\), K\(^+\), ATP-ase. Dexamethasone is absorbed in the normal spinal cord also, suggesting the changes in enzyme activity in sham animals.

Verapamil in the doses given for one week partially reverses the enzyme activity of injured segment. Verapamil has been described to inhibit calcium accumulation into rabbit hippocampus during ischemia (Hagberg et al., 1984) and the drug crosses damaged blood brain barrier during reflow (Chan et al., 1984). Verapamil is a phenylalkene with calcium channel blocking and \(\alpha\)-receptor binding activity at low concentrations (Triggle, 1987), which also exhibits activity on sodium and potassium channels at high concentrations (Atlas, 1981).

**Lysosomal enzymes:** The present studies, show that lysosomal enzymes are affected by spinal cord injury. There was a significant increase in the release of lysosomal enzymes, \(\beta\)-D-hexosaminidase and \(\alpha\)-L-fucosidase after one week spinal cord injury. The high levels of hexosaminidase and fucosidase in CSF would suggest a leakage of these enzymes into the extracellular space in spinal cord ischemia. Previously, there have been studies which suggest that lipid peroxidation causes the rupture of lysosomes and consequent release of lysosomal hydrolytic enzymes in the ischemic monkey brain basal ganglia (Nagarajan et al., 1988). Lysosomal enzyme release has been
implicated in the pathogenesis of late phases of spinal nerve tract damage in feline experimental spinal cord injury (Kakari, 1974). A sudden impact injury produces destruction of grey matter, impaired micro circulation, vasogenic edema, axonal shrinkage, massive hemorrhage and tissue necrosis. The increase in lysosomal enzymes may be due to inflammatory processes in the spinal cord following trauma.

Both verapamil and dexamethasone were found to have a protective effect on lysosomal enzyme release in this study. These drugs probably act by blocking some of the autocatalytic pathophysiologic pathways. There are reports on the efficacy of steroids in stabilizing lysosomal membranes (Weissmann, 1964).

Phospholipids: Our data presents a decreased total phospholipid and individual phospholipids, phosphatidyl ethanolamine and phosphatidyl choline at 24 hours injury in rat spinal cord. A similar phospholipid decrease was observed by Segler-Stahl et al., (1985) at 3 hours post injury following impact trauma to miniature pigs, in terms of wet weight and Faden et al., (1987) also found a similar decrease at 24 hours after impact trauma to rats. Demediuk et al., (1985a) found no statistically significant changes in total phospholipid levels in cat spinal cord following compression injury, when expressed per sphingomyelin content.
Under normal conditions, phospholipid composition is controlled through a complex interplay of energy dependant reaction including a rapid fatty acid side chain deacylation-reacylation cycle, head-group base exchange, and slower de novo biosynthesis (Dawson, 1973; Sun et al., 1979; Stubbs & Smith, 1984). The decrease in phospholipids can be due to increased hydrolysis, decreased reacylation and decreased de novo synthesis. Increase in Ca\(^{2+}\) activates phospholipases A and C and through calcium-dependent binding of calmodulin to phospholipase A\(_2\) (Moskowitz et al., 1984). Following spinal injury, energy stores are depleted, which diminish the rates of FFA reacylation and de novo biosynthesis of phospholipids (Dawson, 1973, Sun et al., 1979). The decrease in phospholipid seen at 24 hours, can be due to secondary injury processes. Changes in lipid metabolism following spinal cord trauma may damage cells and affect physiological processes. Membrane lipid changes could account for the rapid decrease in extracellular Ca\(^{2+}\) (Young et al., 1982, Stokes, 1983) and the decrease in total Mg\(^{2+}\) that are seen after spinal injury (Lemke et al., 1987).

**NEUROLOGIC FUNCTION**

An improved neurologic score was observed with dexamethasone and verapamil in monkeys and rats. Clinical grading, reflects the degree of long tract degeneration after injury. In the rat, motor function is carried out primarily
by the rubrospinal (flexor motoneurons), lateral vestibulospinal (extensor motoneurons), and corticospinal tracts (Tracey, 1985). It is the loss of neural tissue particularly in the gray matter, at the end of histopathological events that should be considered as the cause of paraplegia (Fairholm, 1971; Gelfan, 1956; Tarlov, 1972). Morphological results demonstrate that some but not all of the tracts were severely damaged by photochemically induced infarction (Prado et al., 1987). Therefore motor recovery might be the result of undamaged motor tracts taking over functions disrupted by the insult. In rats an improved neurological function was shown by the inclined plane technique, with verapamil and dexamethasone. The loss of motor function that follows traumatic spinal cord injury appears to result partly from secondary, delayed injury caused by the release of endogenous auto destructive factors (Faden, 1983). Dexamethasone in the doses given was found to be effective in improving functional outcome in both primates and rats. Dexamethasone prevents loss of axonal conduction and reflex activity, following acute spinal cord injury (Naimiento, 1979). Verapamil also found to improve neurological outcome in both monkeys and rats. Verapamil prevents calcium toxicity and thereby inhibits the further damage to membranes. The other drugs given were found to be ineffective in improving neurologic function. Naloxone with a bolus injection was ineffective in this study, suggesting the earlier reports
that, the beneficial effect of naloxone is dose related (Faden, 1984). The dose administered may be inadequate to bring any changes in this study.

HISTOPATHOLOGICAL CHANGES

Histopathological studies show that this model of injury, produced hemorrhagic necrosis, seen more confined to grey matter spreads to white matter slowly. Pathological changes are either primary or secondary. The direct primary traumatic changes, disruption of neurons, glial cells, myelin and vascular system, are seen directly under the impact. The secondary pathological changes are hemorrhage and edema. The pathological changes observed after one week in this study are focal hemorrhage and spongiosis in gray matter as well as white matter. There was ischemic cell change, pallor of neuropil, disruption of grey matter, chromatolysis, cavitation and swollen axons. Our data failed to show any pathologic differences in the spinal cords between treatment (dexamethasone and verapamil) groups and control saline group. This may be due to the insensitive microscopic techniques used in predicting functional neurologic recovery after spinal injury.

PHARMACOKINETICS OF VERAPAMIL

This kinetic study shows that, verapamil in the doses given crosses blood brain barrier in spinal cord injured
animals. Verapamil is a calcium antagonist, that specifically blocks the entry of $\text{Ca}^{2+}$ into excitable cells through slow calcium channels. (Kohlhardt et al., 1972). Tissue concentration electrophysiologic studies indicate that the ability of verapamil to block calcium dependent membrane phenomena is apparent when measurable quantities of the drug have vanished. Thus concentration of physiologically active material may be below the level of detection. In this study very negligible levels of verapamil was seen in both sham and experimental spinal cord tissues. Verapamil kinetics have been studied extensively and wide variation has been reported for elimination kinetics and clearance. (Eichelbaum 1979,1980; Woodcock, 1980).

Plasma concentrations above $\approx 100$ nanograms/ml were associated with improvement and peak verapamil concentrations upto 900 nanograms were found to be without any adverse effects (Hamann et al, 1984). Verapamil found in the CSF may be unbound verapamil or may be protein bound as the blood brain barrier was damaged in spinal cord injury, so the protein bound form crosses the blood brain barrier.

To summarize, verapamil and dexamethasone were found to be effective in the weight-drop model and rat compression model. Our data shows an improvement in neurologic, and enzyme changes with both dexamethasone and verapamil. No pathologic
changes could be detected. Naloxone in a bolus injection, improved the post traumatic hypotension, but failed to show any neurologic improvement suggesting high doses may be required for its beneficial effect in spinal injury. Dimethyl sulfoxide in the doses given has no beneficial effect in this model of spinal injury, may be the doses given are toxic. Nifedipine also failed to show any beneficial effect in this study because of its solvents, propylene glycol, and ethyl alcohol. Dipyridamole also found to be ineffective in this model.