Spinal cord energy metabolism following compression trauma to the feline spinal cord

DOUGLAS K. ANDERSON, PH.D., EUGENE D. MEANS, M.D.,
THOMAS R. WATERS, B.A., AND CRATE J. SPEARS, B.A.

Departments of Neurology and Physiology, Veterans Administration Medical Center, and University of Cincinnati College of Medicine, Cincinnati, Ohio

The purpose of this study was to determine the spinal cord metabolic state for 24 hours after compression trauma to the feline spinal cord. Cats were anesthetized with pentobarbital and injured by placing a 190-gm weight on the spinal cord for 5 minutes. Biochemical analysis of the injured segment revealed a significant depletion in the levels of adenosine triphosphate (ATP), phosphocreatine (P-creatine), and total adenylates for the entire 24-hour recovery period. Glucose levels initially declined, but by 1 hour had normalized, and at 8 and 24 hours were significantly supranormal. The lactate/pyruvate ratio and tissue lactate concentrations increased four and five and a half times, respectively, for the first 4 hours after injury. Between 8 and 24 hours, lactate levels remained elevated, whereas the lactate/pyruvate ratio declined to control levels as the result of a significant rise in the tissue pyruvate concentration. This sequence of metabolic changes suggested that metabolism was probably not homogeneous throughout the injured segment, and that tissue metabolic rate was depressed for the initial 4 hours after trauma then increased in metabolically active tissue for the remainder of the 24-hour recovery period. This model of spinal cord trauma results in a severe, prolonged ischemia and metabolic injury to the affected tissue. Whether these metabolic changes result from or cause the tissue damage and irreversible paraplegia associated with this type of spinal cord injury remains to be determined.

KEY WORDS - spinal cord injury - compression trauma - energy metabolism - spinal cord compression

RECENT studies have demonstrated that one consequence of trauma to the spinal cord is a reduction in blood flow to the injured tissue of variable onset, duration, and magnitude. Although numerous investigations have confirmed that cerebral ischemia produces a tissue lactic acidosis concomitant with diminishing high-energy phosphate reserves, only three reports have been directed at determining the status of spinal cord energy metabolism following trauma. The purpose of this study was to ascertain the effects of spinal cord compression trauma on feline spinal cord energy metabolism, that is, to determine the pattern of changes in the tissue concentration of selected glycolytic substrates and high-energy phosphates for 24 hours after compression trauma to the spinal cord.

Materials and Methods

A total of 60 immunized and conditioned female mongrel cats, ranging in weight from 2.3 to 3.8 kg, were used for this study. All animals were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg) and intubated. Arterial samples for blood gas and pH analysis were secured and arterial blood pressure was monitored, both from the midthoracic aorta. A femoral vein was also cannulated to administer supplemental doses of anesthesia and other drugs as necessary. The cats were paralyzed with succinylcholine chloride (1 mg/kg), and ventilated with positive pressure using a mixture of room air, 95% O₂, and 5% CO₂. This gas mixture was necessary to simultaneously maintain the desired arterial partial
compression trauma for the injured group, spinal cord tissue was frozen in situ with an intact blood supply by pouring liquid nitrogen onto the exposed vertebral column and spinal cord. Under continued liquid nitrogen irrigation, any remaining bone was chiseled away, and the desired segment of spinal cord was removed intact after carefully separating each end from the remainder of the spinal cord. Details of our in situ freezing technique have been published previously. Spinal cord pieces (weighing about 25 to 35 mg) containing both white and gray matter from directly beneath the L-2 laminectomy site in control animals, or through the center of the traumatized L-2 segment in injured cats, were pulverized under liquid nitrogen. Spinal cord tissue was extracted using the method described by Gorell, et al., with certain minor modifications. The frozen powders were lightly agitated for 15 minutes at -40°C with three volumes of 99% methanol-0.1N-HCl. The samples were transferred to an ice water bath (0°C), diluted with 20 volumes of 0.44N-HCI04 containing 5mM EDTA (ethylene-diaminetetraacetic acid) and repeatedly mixed for 15 minutes. After centrifugation at 12,000 G for 15 minutes, the supernatant was removed and neutralized with 1.29N-KOH-0.4M-KCI-0.4M imidazole. After a second centrifugation, the neutralized supernatant was analyzed for phosphocreatine (P-creatine), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), lactate, pyruvate, and glucose by means of the enzymatic, fluorometric methods of Lowry and Passonneau.

The concentrations of all measured metabolites are expressed in mmol/kg wet tissue. The adenylate energy charge (EC) was:

\[ EC = \frac{[\text{ATP}] + 0.5[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]} \]

and total adenylates were \( \Sigma \text{ATP} + \text{ADP} + \text{AMP} \). These values were calculated as an index of the energy status of the tissue. The lactate/pyruvate ratio was calculated as an estimate of the oxidation-reduction state of the tissue. Results from experimental groups were compared by one-way analysis of variance. Further comparisons between pairs of groups were made by Duncan's multiple-range test. A probability of 0.05 was set as the minimum level of significance.

Results
The data from this study are displayed in Figs. 1 and 2. Compression of the spinal cord for 5 minutes with a 190-gm weight resulted in reductions of approximately 70% for ATP and 78% for P-creatine within the first 15 minutes after trauma (Fig. 1 upper). Thereafter, the concentrations of these metabolites were essentially unchanged from this level for the remainder of the 24-hour period after injury, although there appeared to be a tendency for P-creatine concentrations to rise at 8 and 24 hours. Tissue levels of ADP...
were significantly depressed by 4 hours after injury, remaining at this level through 24 hours (Fig. 1 center). Concentrations of AMP were essentially doubled for the first 4 hours after injury; then returned to preinjury levels at 8 and 24 hours (Fig. 1 center). The sum of the adenine nucleotides fell 57% within the first 15 minutes following compression, then declined an additional 8% to 10% over the next 24 hours (Fig. 1 lower). The calculated energy charge was significantly reduced by an average of 17% for the first 4 hours after compression. Although still significantly subnormal, the energy charge had increased slightly by 8 hours, and by 24 hours was only 7% below control.

Tissue glucose concentrations were 59% of control 15 minutes after spinal cord compression (Fig. 2 upper). At 1 and 4 hours, tissue glucose had recovered to preinjury levels. Between 4 and 8 hours after injury, tissue glucose levels increased significantly, achieving values 70% above control by 24 hours. The pyruvate concentration in injured spinal cord tissue was significantly elevated by 15 minutes following compression (Fig. 2 upper). Between 4 and 24 hours, there was an additional increase in pyruvate levels, reaching values 2.3 times higher than control by 24 hours. Tissue lactate acid concentration was elevated a constant five and a half times for the first 4 hours after injury (Fig. 2 center). At 8 and 24 hours, tissue lactate was still significantly elevated above control, although the lactate concentrations at these two time periods tended to be lower than those between 15 minutes and 4 hours after injury. The lactate/pyruvate ratio was elevated an average of fourfold for the first 4 hours following compression trauma (Fig. 2 lower). After 4 hours, the lactate/pyruvate ratio declined to a value at 24 hours that, while still elevated twofold, was not significantly different from control. Since lactate levels were not substantially reduced at 8 and 24 hours, the decline in the lactate/pyruvate ratio was due to the rise in tissue pyruvate at these two time periods.

Discussion

To better interpret the results of this study, it is appropriate to briefly survey previous data from this and other laboratories concerning microvascular perfusion, blood flow, and recovery after compression trauma to the spinal cord.

Using a colloidal carbon perfusion technique, we demonstrated that, for the first 4 hours following compression, feline spinal cord microvascular perfusion was reduced 75% to 83% in gray matter and 55% to 60% in white matter. Microperfusion continued to fall, and at 8 and 24 hours was approximately 2% and 8% of control in gray and white matter, respectively. Although microvascular perfusion studies are not direct measurements of blood flow, a lack of vascular filling implies a lack of blood flow. These data suggest that one consequence of spinal cord compression is a prolonged oligemia that becomes extremely severe between 4 and 8 hours after compression. Results from Tator’s laboratory have confirmed that an extended oligemia is a major complication of spinal cord compression. These workers compressed the upper thoracic spinal cord of albino rats for 5 minutes with an extradural clip that produced a force of 180 gm. Spinal cord blood flow (SCBF) was measured with the 14C-antipyrine autoradiographic technique. Blood flow to the compressed segment was completely obliterated during the 5-minute compression period. After release of the clip, SCBF remained severely depressed, averaging only 3% of control in white matter and 1% of control in gray matter for the duration of a 24-hour recirculation period. Thus, compression of the spinal cord with a force of 170 to 190 gm for 5 minutes results in a complete ischemia during the compression period, then a severe incomplete ischemia for at least 24 hours after injury. In our laboratory, a 190-gm weight placed on the feline spinal cord for 5 minutes results in a paraplegia that lasts for at least 2 months. Few animals have been
followed for longer periods of time, but those that were observed demonstrated no additional improvement of neurological function. Thus, this magnitude of spinal cord compression causes irreversible paraplegia in cats.

The present studies demonstrate that compression of the spinal cord produces a marked depletion of high-energy phosphate reserves and a lactic acidosis as the result of ischemia.\textsuperscript{21,26,27} Similar metabolic responses for the ischemic brain\textsuperscript{14,15,17,18,22,25} and spinal cord\textsuperscript{2,23,35} have previously been reported. However, as stated by Nordström and Siesjö,\textsuperscript{25} assessment of metabolic changes in incomplete ischemia is problematic, as these changes can be variable and unexpected, and may result from a lack of homogeneity of blood flow and metabolism. Thus, much of these data are open to interpretation.

Metabolic changes in injured spinal cord tissue occurred in two stages during the 24-hour postinjury period. While total adenylates and tissue concentrations of ATP, P-creatine, and lactate were essentially constant for 24 hours, the sequence of changes in the lactate/pyruvate ratio, in the energy charge, and in the tissue levels of pyruvate and glucose were different for the first 4 hours than between 4 and 24 hours after injury.

The decline in spinal cord glucose concentration seen 15 minutes after injury is probably due to utilization of tissue glucose stores during a period of diminished glucose delivery to the tissue occurring when the spinal cord is compressed for 5 minutes. The rise in glucose between 15 minutes and 4 hours after injury is likely replenishment of tissue supplies as a result of the residual SCBF.\textsuperscript{21,28,27} Plateauing tissue lactate levels for the first 4 hours following injury (in the face of increasing tissue glucose concentrations) might suggest removal of a portion of the formed lactate.\textsuperscript{35} Alternatively, the static lactic acid levels may be due to a decreased or limited metabolic rate in the injured tissue stemming from several possible mechanisms. First, the rate of anaerobic glycolysis may have been restricted due to a lowered tissue pH that resulted from the accumulated lactic acid.\textsuperscript{7,31} Second, cellular swelling secondary to an intracellular acidosis resulting from the accumulated lactate,\textsuperscript{15,22} and/or loss of the metabolic energy required for maintenance of ionic gradients across cell membranes,\textsuperscript{7,11,28} might depress the tissue metabolic activity and rate of anaerobic glycolysis. Third, since it is well known that barbiturates depress cerebral metabolism,\textsuperscript{3,30,31} the use of pentobarbital as the anesthetic for this study may have suppressed spinal cord tissue metabolic rate for the initial 4 hours of the period after injury. In the process, the injured tissue may have been protected from more severe damage, as suggested by Walker, \textit{et al.}\textsuperscript{24} Further studies with other anesthetic agents are needed to clarify this point.

A third interpretation is that the unchanging tissue lactate levels might reflect a lack of homogeneity of metabolism, that is, the presence of both anaerobic and aerobic areas within the injured spinal cord segment. For example, if aerobic metabolism were minimal, one might expect steadily rising tissue lactate levels and lactate/pyruvate ratios to have occurred with the increasing tissue glucose concentration, as previously demonstrated for the oligemic brain.\textsuperscript{25} Thus, the lack of change in tissue lactate may reflect an averaging of these anaerobic and aerobic areas.

Between 4 and 24 hours, tissue levels of glucose and pyruvate became supranormal, the lactate/pyruvate ratio fell to control levels, and there was a slight rise in the energy charge. The increase in tissue glucose and pyruvate levels suggested a stimulation or release of the glycolytic rate from its previously depressed state. Decline in the lactate/pyruvate ratio implies that oxidative pathways were operative. The mechanism(s) responsible for the increased metabolic rate are not known. Possibly the effects of pentobarbital on metabolism were diminished after 4 hours. In addition, regional differences in cellular swelling might exist. In those areas where edema was minimal or diminishing, some recovery of metabolic rate would be expected.\textsuperscript{35} Although the injured segment was markedly oligemic,\textsuperscript{21,28,27} the remaining SCBF must be adequate to subserve some oxidative metabolism. Welsh, \textit{et al.},\textsuperscript{36} recently demonstrated the presence of oxidized regions in severely ischemic brains, and suggested that this occurred as the result of a "patchy perfusion" that was below the detection limits of their method for measuring blood flow. The rise in tissue glucose levels we observed between 4 and 24 hours may have been due to increased perfusion to microregions within the injured segment, perhaps coupled with an augmented glucose transport from blood.\textsuperscript{9}

Two theories have previously been advanced to explain the prolonged depression of the adenylate pool and tissue ATP levels in the oligemic cerebrum. First, during ischemia, ATP is dephosphorylated to ADP and AMP, and the size of the adenine nucleotide pool is diminished secondary to the degradation of AMP to inosine monophosphate (IMP) and adenosine.\textsuperscript{25} Since many of the AMP metabolites are diffusable and resynthesis of adenine nucleotides is slow, the size of the adenylate pool may be subnormal for extended periods.\textsuperscript{30} Consequently, adequate resynthesis of ATP may be prevented by insufficient levels of ADP and AMP.\textsuperscript{24} Second, it has been suggested that lack of ATP resynthesis and persistent depression of total adenylates reflect irreversible tissue damage.\textsuperscript{14,24,36} Histological studies from our laboratory after compression injury of the spinal cord showed hemorrhagic necrosis of gray and white matter and prominent ischemic nerve cell change.\textsuperscript{18,20} These structural alterations are consistent with the biochemical changes detected in the present study. Thus, the injured segment may present a spectrum of tissue injury ranging from normal to necrotic. Consequently, the
sequence of metabolic changes seen in these experiments may reflect an averaging of these necrotic, anaerobic, and aerobic areas, as alluded to previously. For the initial 4 hours, anaerobic metabolism likely predominated. However, between 4 and 24 hours there appeared to be an increasing percentage of oxidative metabolism in the remaining metabolically viable tissue. From these studies it is not possible to determine whether prolonged depletion of high-energy phosphates was the result of or caused tissue damage. The only statement that can be made with certainty is that associated with these metabolic changes are tissue necrosis and an irreversible paraplegia. Additional studies aimed at restoring postinjury SCBF and metabolism should aid in elucidating the role of a deranged energy metabolism in production of cellular damage.

Ischemia of 30 to 60 minutes' duration is generally required to cause the same magnitude of postischemic metabolic injury and oligemia in brain tissue that we created in spinal cord with 5 minutes of mechanical compression. This suggests that mechanical deformation in some manner injures the spinal cord vasculature, rendering it incapable of sustaining blood flow. Perhaps compression of spinal cord tissue causes a persistent vasospasm that severely limits recirculation once the compression is relieved. Studies directed at explaining the mechanism(s) underlying this mechanically induced vascular collapse are required.

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References

22. Michenfelder JD, Suntif TM: Cerebral ATP and lactate levels in the squirrel monkey following occlusion of the middle cerebral artery. Stroke 2:319-326, 1971

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Address reprint requests to: Douglas K. Anderson, Ph.D., Service of Neurology, Veterans Administration Medical Center, 3200 Vine Street, Cincinnati, Ohio 45220.