

TISSUE CALCIUM LEVELS FOLLOWING SPINAL CORD TRANSECTION IN RATS

John B. GELDERD and H. Wayne SAMPSON

Department of Anatomy, College of Medicine, Texas A and M University
College Station, Texas 77843, USA

Key words: calcium, spinal cord transection, atomic absorption spectrophotometry

Abstract. Adult rats were subjected to midthoracic spinal cord transections. Three segments of spinal cord, each approximately 5 mm in length, were removed from each animal at intervals from 5 min to 48 h postlesion; one from the lesion site and one each immediately rostral and caudal to the transection. Total tissue calcium concentrations ($[Ca]_t$) for each spinal cord segment were determined using atomic absorption spectrophotometry and compared to control segments from untransected animals. $[Ca]_t$ levels in the segment at the lesion site was significantly elevated above control values at 30 min post-lesion, but decreased to control levels by 1 h. All other segments remained at control levels for the duration of the postlesion period. The rapid rise and fall of $[Ca]_t$ at the lesion site differs from spinal cord contusion studies in which $[Ca]_t$ remains at elevated levels for extended periods. It is postulated that the "open" transection injury permits the rapid clearance of calcium from the injury site.

INTRODUCTION

Until it was reported in 1973 (22) that high calcium concentrations increased axonal degeneration following transection of rat nerve fibers *in vitro*, tissue calcium levels were virtually ignored as a significant factor in the pathophysiology that occurs following mammalian spinal cord injury (9, 17, 18, 20, 27, 28). Since then, a number of investigators

have implicated calcium as a mediator for cell injury and necrosis in a variety of biological systems (21, 26, 32), including the destruction of adjacent neuropil that occurs following spinal cord injury in mammals (4 - 7, 10, 15, 16, 23 - 25, 29 - 31).

Intracellular calcification of axons following spinal cord injury in rats was first reported by Balentine, et al. (6), using electron microscopy and x-ray microanalysis. Clusters of calcium phosphate crystals, in the form of needles, were seen surrounding various intraaxonal organelles, particularly mitochondria. These crystals were seen as early as 30 min following spinal cord contusion and appeared to peak within 4 h of injury. Using both light and electron microscopy with x-ray microanalysis, the above initial findings were subsequently confirmed in accidental human spinal cord trauma and experimental spinal cord contusion in cat and monkey (3). In addition to axonal degeneration, calcium has also been linked to the vesicular degeneration of myelin which occurs following spinal cord injury or calcium-induced myelopathy (2, 5, 8).

Subsequent studies, using a spinal cord contusion model, revealed a sustained and significant drop in extracellular calcium within 5 min of injury (7, 10, 23 - 25, 30). Recovery of extracellular calcium to preinjury levels was dependent on the distance from the injury site. In a quantitative tissue calcium study using atomic absorption spectrophotometry (AAS) following spinal cord contusion, the authors reported a sustained and significant increase in total tissue calcium concentration ($[Ca]_t$) for up to 7 days postlesion (16). In a similar, more recent study of $[Ca]_t$, the authors reported that the $[Ca]_t$ gain at the injury site within 1 h approximated the $[Ca]_t$ loss in the surrounding cord, leading them to postulate that increased $[Ca]_t$ at the lesion site was derived from adjacent spinal cord and sequestered in organic phosphates, causing a deep calcium sink at the contusion site (29).

Anatomical studies of spinal cord contusion appear to coincide with chemical studies since severe central hemorrhagic necrosis occurs within 4 h following injury, destroying adjacent gray matter at the contusion site with the resultant formation of spinal cord cavitations (4, 5). Conversely, spinal cord transection studies reveal a more leisurely pace for secondary destruction of surrounding cord neuropil and the subsequent development of cavitations. In previous studies in our laboratory (11 - 14, 19), spinal cord transection in rats resulted in the formation of microcysts in adjacent spinal neuropil during the initial 7 postoperative days. By 15 days, many of these microcysts had coalesced to form small, multiple cavitations at the injury site. However, viable gray matter, containing neurons, could still be seen immediately adjacent to the scar that formed at the transection site. Moreover, axons from the adjacent neuro-

pil could be seen penetrating short distances into the scar at 15 days postlesion. By 30-45 days, large cavitations formed at the expense of spinal cord neuropil. These cavitations continued to expand with time until they occupied over 90% of the cross-sectional area of the adjacent spinal cord by 60 - 90 days postlesion.

Since dissolution of adjacent spinal neuropil proceeds at a slower pace in transection injuries versus contusion injuries, and all spinal cord injury studies involving $[Ca]_t$ levels have been done following contusion injuries, it was decided that an assessment of $[Ca]_t$ following spinal cord transection would be appropriate to further clarify the role of calcium in mediating the initial destruction and progressive necrosis of neuropil that occurs following spinal cord injury.

MATERIAL AND METHODS

Experimental animals. Twenty-four adult female Long-Evans hooded rats served as experimental animals in this study.

Surgical procedure. Following anesthetization with chloral hydrate (400 mg/kg i.p.), the back was shaved and the skin swabbed with alcohol. A midline incision was made in the midthoracic region with a scalpel and the back musculature dissected free of the vertebral column. Under an operating microscope, a laminectomy was performed at the T₆ vertebral level and the spinal cord transected with a No. 12 scalpel. Hemostasis was achieved with applications of Gelfoam and the musculature and skin sutured shut in layers.

Animal care, survival periods and tissue acquisition. Following surgery, animals were separated into 7 groups of 3 each for postoperative survival periods of 5, 15 and 30 min; 1, 12, 24 and 48 h. Animals surviving for 24 and 48 h received manual bladder expressions twice daily. At the end of each survival period, each animal was anesthetized as described above. The laminectomy was extended rostral and caudal to the transection site and 3 segments of spinal cord, each approximately 5 mm in length, were removed as follows: one segment encompassing the transection site, one segment rostral, and one segment caudal to the transection site. In addition, a control group of 3 untransected animals were anesthetized. In each control animal, 3 segments of spinal cord were removed from the same location as described for the transected animals. Following tissue removal, all animals were killed by over-doses of ether.

Mineral analysis. Spinal cord segments were weighed and rapidly frozen until analysis. They were then ashed in a muffle furnace at 600°C for 16 hrs and the ash from each spinal cord segment was dissolved in

5 ml of 1% LaCl_3 in 0.1 N HCl. Calcium levels were determined by AAS (Varian AA-1275), using a nitrous oxide-acetylene fuel mixture. Each sample was tested 3 times and averaged. Statistical analysis of the data was accomplished by using ANOVA.

RESULTS

The results of $[\text{Ca}]_t$ are summarized in Fig. 1. Calcium levels in the transected spinal cords were significantly elevated only at the site of lesion at 30 min following transection. This maximum calcium level of 962 $\mu\text{g}/\text{gm}$ wet weight corresponds to a statistically significant ($p < 0.05$) three-fold increase as compared to the 330 $\mu\text{g}/\text{gm}$ in non-transected control spinal cords. Although $[\text{Ca}]_t$ in spinal segments rostral to the transection site were either slightly greater or less than controls throughout the postoperative periods, there were no statistically significant differences in $[\text{Ca}]_t$ between controls and rostral or caudal spinal cord segments.

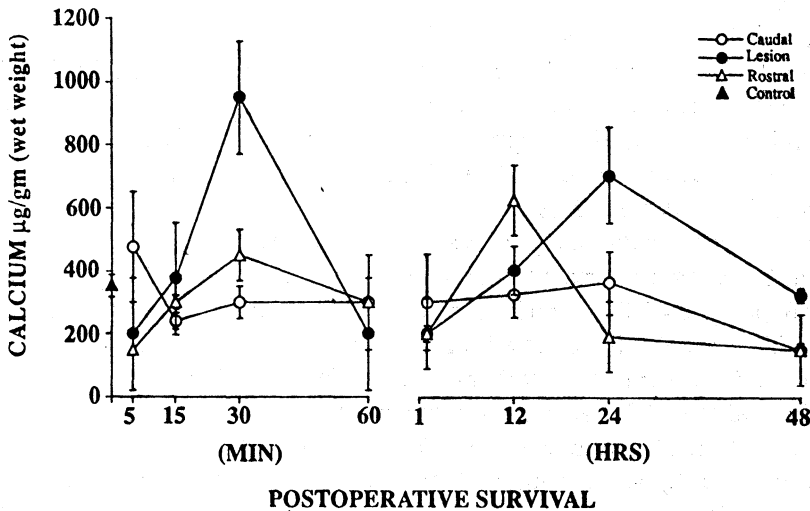


Fig. 1. Summary of quantitative $[\text{Ca}]_t$ data. Each data point represents triplicate measurements on 3 animals, using atomic absorption spectrophotometry. Vertical bars = SEM.

DISCUSSION

The results of this study indicate there is a rapid influx of calcium into the lesion area within 15 min of spinal cord transection. This in-

crease in $[Ca]_t$ reaches its peak and becomes statistically significant by 30 min postlesion. By 1 h, however, $[Ca]_t$ at the lesion site is reduced to control levels and remains there for the duration of the study. Although the initial phase of our results parallel two other AAS studies that used a contusion model, the results diverge quickly. The two other studies reported significant $[Ca]_t$ increases at the contusion site within 45 min or 1 h (16, 29). However, in both contusion studies, $[Ca]_t$ continued to increase with time; in one case for the duration of the postlesion period of 3 h (19), and in the other for 8 h after which $[Ca]_t$ remained approximately 4 times that of controls for the duration of the 7 day postlesion period (16).

The specific reasons for the disparity in $[Ca]_t$ results between transection and contusion injuries require further experimentation. However, there are some plausible explanations for these differences based on previous studies and the basic mechanics of the 2 types of lesions. Contusion lesions typically do not disrupt the overlying meninges, but instantly compress and release the neuropil within the meninges, causing rapid bidirectional movements and shearing forces within the neuropil. These forces cause significant functional and physical disruption of delicate cellular membranes, permitting the intracellular accumulation of calcium (5, 7, 10, 25). Moreover, microvasculature within the cord in the region of the contusion is physically disrupted, resulting in hemorrhage and edema within the injured neuropil (4). Since little expansion occurs because of the intact meninges, there is an increase in pressure within the injured neuropil. This increase in intraparenchymal pressure further compromises blood supply, restricts the flow of cerebrospinal fluid (CSF) and hampers the movement of accumulated intracellular calcium, thus probably contributing to the sequestration of inorganic phosphates and the subsequent precipitation of calcium in the form of hydroxyapatite crystals, which then causes necrosis and cell death at the lesion site. Conversely, typical experimental transection injuries to the spinal cord are accomplished with a scalpel and result in a relatively clean cut through the meninges, all axons, neuronal and glial soma, blood vessels and central canal at the transection site. This, in turn, results in massive hemorrhage and leakage of CSF, axoplasm and extracellular fluids into the surrounding tissues. Since a transection is an open injury, the initial influx of calcium may be diluted and transported from the transection site by the combination of hemorrhage and extravasation of intracellular and extracellular fluids, resulting in a rapid return of $[Ca]_t$ to normal levels.

It should also be pointed out that factors other than calcium accumulation have been strongly implicated in the pathophysiology that occurs

following spinal cord trauma. These include ischemia, acidosis, free radical formation, lipid peroxidation and enzymatic activity (5, 7, 10, 25). However, it appears that calcium may act as a mediator or play a major role in all of these proposed mechanisms for cell necrosis and death following spinal cord trauma (5).

In conclusion, it appears that sufficient evidence exists to implicate calcium as a causative factor in the rapid onset of cell death at the lesion site in contusion injuries. However, the pathophysiological role of calcium following experimental spinal cord transection may be limited due to the rapid clearance of calcium from the injury site within 1 h following transection. Moreover, the role of calcium in the subsequent secondary destruction of adjacent neuropil is equivocal, since $[Ca]_i$ in adjacent neuropil remains at control levels for the duration of the relatively short postoperative periods that have been reported following either spinal cord transection, as in the present study, or spinal cord contusion (16, 29). Long-term studies, combining anatomical and chemical techniques, are required to establish whether calcium assumes a substantive role in the subsequent progressive destruction of adjacent neuropil following spinal cord injury.

The authors wish to thank Mrs. Nadine Stuth for her excellent technical skills in atomic absorption spectrophotometry and Ms. Sherrie Hughes for her typing skills and her uncanny ability to read our illegible handwriting.

REFERENCES

1. BALENTINE, J. D. 1978. Pathology of experimental spinal cord trauma. I. The necrotic lesion as a function of vascular injury. *Lab. Invest.* 39: 236 - 253.
2. BALENTINE, J. D. 1978. Pathology of experimental spinal cord trauma. II. Ultrastructure of axons and myelin. *Lab. Invest.* 39: 254 - 266.
3. BALENTINE, J. D. 1980. Axonal calcification in spinal cord injury of humans, monkeys and cats. *Lab. Invest.* 42: 99.
4. BALENTINE, J. D. 1983. Calcium toxicity as a factor in spinal cord injury. *Surv. Synth. Pathol. Res.* 2: 184 - 193.
5. BALENTINE, J. D. 1988. Spinal cord trauma: in search of the meaning of granular axoplasm and vesicular myelin. *J. Neuropathol. Exp. Neurol.* 54: 77 - 92.
6. BALENTINE, J. D. and SPECTOR, M. 1977. Calcification of axons in experimental spinal cord trauma. *Ann. Neurol.* 2: 520 - 523.
7. BANIK, N. L., HOGAN, E. L., POWERS, J. M. and SMITH, K. P., 1986. Proteolytic enzymes in experimental spinal cord injury. *J. Neurol. Sci.* 73: 245 - 256.
8. BANIK, N. L., HOGAN, E. L. and HSU, C. Y. 1987. The multimolecular casca-

- de of spinal cord injury: studies of prostanoids, calcium, and proteinases. *Neurochem. Pathol.* 7: 57 - 77.
9. DOHRMANN, G. J. 1972. Experimental spinal cord trauma: A historical review. *Arch. Neurol.* 27: 468 - 473.
 10. FADEN, A. I. 1987. Pharmacotherapy in spinal cord injury: A critical review of recent developments. *Clin. Neuropharmacol.* 10: 193 - 204.
 11. GELDERD, J. B. 1983. Hyperbaric oxygen and dimethyl sulfoxide therapy following spinal cord injury. In C. C. Kao, R. P. Bunge and P. J. Reier (ed.), *Spinal cord reconstruction*. Raven Press, New York, p. 245 - 259.
 12. GELDERD, J. B., FIFE, W. P., BOWERS, D. E., DESCHNER, S. H. and WELCH, D. W. 1983. Spinal cord transection in rats: The therapeutic effects of dimethyl sulfoxide and hyperbaric oxygen. *Annals NY Acad. Sci.* 411: 218 - 233.
 13. GELDERD, J. B., MATTHEWS, M. A., ONGE, M. F. ST. and FACIANE, C. L. 1980. Qualitative and quantitative effects of ACTH, piromen, cytoxan and isobutyl-2-cyanoacrylate treatments following spinal cord transection in rats. *Acta Neurobiol. Exp.* 40: 489 - 500.
 14. GELDERD, J. B., WELCH, D. W., FIFE, W. P. and BOWERS, D. E. JR. 1980. Therapeutic effects of hyperbaric oxygen and dimethyl sulfoxide following spinal cord transection in rats. *Undersea Biomed. Res.* 7: 305 - 320.
 15. HAPPEL, R. D., BANIK, N. L., BALENTINE, J. D. and HOGAN, E. L. 1984. Tissue calcium levels in CaCl_2 -induced myelopathy. *Neurosci. Lett.* 49: 279 - 283.
 16. HAPPEL, R. D., SMITH, K. P., BANIK, N. L., POWERS, J. M., HOGAN, E. L. and BALENTINE, J. D. 1981. Ca^{2+} -accumulation in experimental spinal cord trauma. *Brain Res.* 211: 476 - 479.
 17. HUGHES, J. T. 1966. *Pathology of the spinal cord*. Year Book Medical Publishers, Chicago, p. 38 - 57.
 18. JELLINGER, K. 1972. Traumatic vascular disease of the spinal cord. In P. J. Vinken and G. W. Bruyn (ed.), *Handbook of clinical neurology*. Vol. 12. Elsevier, New York, p. 556 - 630.
 19. MATTHEWS, M. A. and GELDERD, J. B. 1986. Qualitative studies of reactive events in the site of injury following transection of the spinal cord in the rat. In G. D. Das and R. B. Wallace (ed.), *Neural transplantation and regeneration*. Springer-Verlag, New York, p. 149 - 180.
 20. OSTERHOLM, J. L. 1974. The pathophysiological response to spinal cord injury: the current status of related research. *J. Neurosurg.* 40: 5 - 33.
 21. SCHANNE, F., KANE, A. B., YOUNG, E. E. and FARBER, J. L. 1979. Calcium dependence to toxic cell death: A final common pathway. *Science* 206: 700 - 704.
 22. SCHLAEPFER, W. W. and BUNGE, R. P. 1973. Effects of calcium ion concentration on the degeneration of amputated axons in tissue culture. *J. Cell Biol.* 59: 456 - 470.
 23. STOKES, B. T., FOX, P. and HOLLINDEN, G. 1983. Extracellular calcium activity in the injured spinal cord. *Exp. Neurol.* 80: 561 - 572.
 24. STOKES, B. T., HOLLINDEN, G. and FOX, P. 1984. Improvement in injury induced hypocalcemia by high-dose naloxone intervention. *Brain Res.* 290: 187 - 190.
 25. STOKES, B. T. and SOMERSON, S. K. 1987. Spinal cord extracellular micro-environment: Can the changes resulting from trauma be graded? *Neurochem. Pathol.* 7: 47 - 55.

26. TRUMP, B. F., BEREZESKY, I. K., LAIHO, K. U., OSORNIO, A. R., MERNER, W. J. and SMITH, M. W. 1980. The role of calcium in cell injury, a review. *Scan. Elec. Micro.* 2: 437 - 462.
27. WAGNER, F. C., AND, J. R., BUCY, P. C. 1972. Recent research on spinal cord injury. *Arch. Neurol.* 27: 465 - 467.
28. WOLMAN, L. 1964. The neuropathology of traumatic paraplegia: A critical historical review. *Paraplegia* 1: 233 - 251.
29. YOUNG, W. and KOREH, I. 1986. Potassium and calcium changes in injured spinal cords. *Brain Res.* 365: 42 - 53.
30. YOUNG, W., YEN, V. and BLIGHT, A. 1982. Extracellular calcium ionic activity in experimental spinal cord contusion. *Brain Res.* 253: 105 - 113.
31. YOUNG, W., YEN, V. and BLIGHT, A. 1982. Extracellular calcium activity in experimental spinal injury. *Brain Res.* 253: 105 - 113.
32. ZIMMERMAN, U-J. P. and SCHLAEPFER, W. W. 1984. Calcium-activated neutral protease (CANP) in brain and other tissues. *Prog. Neurobiol.* 23: 63 - 78.

Accepted 1 March 1990