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Neuroprotective Effects of Ketamine in a Rodent Model of Peptide Induced Spinal Cord Injury: Anatomical and Physiological Correlates.

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D.D. Rigamonti, A. Martinez-Arizala, and J.B. Long

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Dynorphin A (1-17) [D Y N] is an endogenous peptide which produces spinal cord ischemia, nociceptive loss and hindlimb paralysis when injected into the lumbar cistern of unanesthetized or halothane anesthetized rats. We initially noted that ketamine (K) when used as the anesthetic agent during routine recording of somatosensory evoked potentials (SEP) preserved the SEP cortical response and affected the neurologic outcome in the previously reported paralytic dose (20 nM, D Y N) was used. Subsequently we reported that K and other N M D A receptor antagonists in the neurologic recovery following D Y N injection. The current study was divided into two experiments. The first, a neuroanatomical study divided into Groups I-IV, were performed in adult Sprague Dawley rats anesthetized with halothane. The i.t. dose of D Y N was 20 m m for all animals. A lumbar subarachnoid (i.t.) injection of saline (I), D Y N alone (20 nM, II), D Y N plus K (4nM, III) and D Y N plus K (2nM, IV). The animals were neurologically evaluated for 72 hours, anesthetized and perfused per cardia with formalin and the lumbosacral cord removed. Paraffin embedded tissue was prepared for histological evaluation using H&E, Nissl, Kluver-Barrera and Bielschowsky techniques in serial cross and horizontal sections.

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The samples were evaluated by three blinded observers and the inter rater agreement and differences between groups were statistically analyzed. Group I animals were neurologically and histologically normal while Group II were paralyzed and exhibited extensive necrosis in the sacral and lower lumbar spinal cord. The injury was characterized by widespread ischemic cill changes, neuronalcell loss and axonal degeneration in the gray andadjacent white matter, with diffuse gliosis and cellular infiltration. Tissue from Group III exhibited injury in the sacralcord and significant in the lumbar enlargement. The animals walked with slight impairment. Group IV animals were not paralyzed and exhibited necrosis in the sacral cord with some preservation of the lumbar enlargement.The second series of physiological experiments were performed in either acute (I,1 hr) or chronic (II, III,72 hr) animals to test the effects of K on DYN exposed spinal cord tissue. Reflex activity recorded and averaged (Nicolet) from ventral roots (L6, S1) was used as an index ofthe effect of DYN or DYN plus K cotreatment. DYN (I) when applied directly to the exposed spinal cord causes complete loss of the late component of the ventral rootresponse within thirty minutes. Ketamine (4 nM) had little or no effect when coinjected during this time period. However, in the chronic state, (72 hrs.,II,III) animals receiving DYN plus K (4nM) maintained the segmental responses . Animals in Group III show greatly reduced or absent late ventral root response. Thus, ketamine improves neurologic, neurophysiologic, and neuropathological outcome in rats treated DYN.

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Neuroprotective Effects of Ketamine in a Rodent Model of Peptide Induced Spinal Cord Injury: Anatomical and Physiological Correlates

D.D. Rigamonti, A. Martinez-Arizala, and J.B. Long

Walter Reed Institute of Research
Washington, D.C.

INTRODUCTION

The dissociative anesthetic, ketamine, first described in a clinical setting by Corssen and Domino (1966), also increases blood flow to the brain following hypovolemic shock in rodents (Idvall *et al.*, 1980; Idvall, 1981) and protects limbic structures during carotid occlusion in gerbils (Marcoux *et al.*, 1988). During spinal cord ischemia produced by aortic occlusion, ketamine increases the duration of ischemia needed to produce hindlimb paralysis in the rabbit (Robertson *et al.*, 1986). A standardized model of spinal cord ischemia in the rat is rather challenging since the collateral circulation is profuse and segmental radicular arteries are interconnected. A novel approach to produce spinal cord ischemia in this species is through intrathecal injection of the endogenous opiate peptides both called dynorphin A (1-13 and 1-17; dyn means powerful). These peptides cause permanent hindlimb paralysis (Przewlocki *et al.*, 1983), decreased spinal cord blood flow and ischemia (Long *et al.*, 1987), and neuropathological changes (Petras and Long, 1989). We have observed that increased doses of dynorphin A (1-13) were needed to produce paralysis and affect the somatosensory evoked responses when injected into animals anesthetized with ketamine for several hours (Rigamonti *et al.*, 1987). Therefore, we began a series of anatomical and physiological experiments to correlate the protective effects of ketamine on the neuropathological and neurophysiological changes induced by intrathecal injection of these peptides.

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METHODS

Anatomical experiments

Adult male Sprague Dawley rats were anesthetized with halothane and suspended in a stereotaxic unit. A parasagittal skin and paraspinous muscle incision exposed the lower lumbar lamina and the fourth lumbar vertebral interspace. Injections were delivered through a 30 gauge needle that was passed between the L4 and L5 vertebra into the underlying spinal subarachnoid space. Animals were divided into 4 experimental groups: Group I, 0.9% NaCl (saline) injected controls; Group II, dynorphin plus ketamine (2 μmol); Group III, dynorphin plus ketamine (4 μmol); Group IV, dynorphin plus saline. The dynorphin A (1-17) dose was kept constant at 20 nmol, an amount previously determined to produce total hindlimb paralysis in halothane anesthetized animals. The rats were reanesthetized at 72 hr postinjection and perfused transcardially with physiological saline followed by a 10% formalin solution for fixation. Perfusion pressure was maintained between 60-80 mm Hg. The spinal cords were dissected from the spinal canal, and a cord segment extending 35 mm rostral from the tip of the conus medullaris was removed. The cord was divided into 6 segments and sectioned either transversely or horizontally. Cross sections were prepared at 3 levels: the sacral cord (10 mm from the termination of the cord), the 6th lumbar segment, and the lumbar enlargement 35 mm from the tip of the coccygeal segments. The intervening cord segments were sectioned horizontally. The tissue was stained according to the methods of Nissl, with hematoxylin and eosin, and with luxol fast blue. Tissue was subsequently evaluated independently by 3 observers blinded to the experimental treatment. Histological scores were assigned using the following criteria, and the lesion rating mean was calculated and plotted using a SAS statistical package:

1. Normal
2. Mild necrosis of the gray matter (less than 30%) with ischemic cell changes and edema
3. Moderate necrosis of the gray matter (less than 60%) with edematous white matter
4. Severe necrosis involving both gray (greater than 90%) and adjacent white matter, edema, cellular infiltration, and glial proliferation

Neurophysiological experiments

Preliminary experiments were designed to record somatosensory evoked potentials (SSEP) following dynorphin A (1-13, i.t. 50 nmol)

exposure. This dose routinely produced hindlimb paralysis in halothane anesthetized animals. Adult male Sprague Dawley rats (N = 20), anesthetized with ketamine and xylazine (70 and 6 mg/kg, respectively) and maintained under anesthesia with the same drugs throughout the 3-4 hr experiment, were mounted in a stereotaxic frame and the skull overlying the somatosensory (SSI) receiving area for the hindlimb exposed. A low impedance silver chloride electrode pair was placed 10 mm rostral to bregma and at the site of maximum cortical response to monitor the somatosensory evoked response following stimulation of the medial and lateral plantar nerves in the contralateral hindlimb. Square wave pulses of 0.1 msec duration were used at a current strength 10 times the threshold for muscle twitch at a frequency of 1 Hz. The cortical response was averaged (N = 300) using standard amplification procedures with a Nicolet Pathfinder. Peak amplitude and latency were measured, either on line or from a plot of the generated analog signal (SSEP). Dynorphin A (1-13) was injected after a baseline SSEP was established and recordings were conducted for 1 hr. Animals were then allowed to recover and the motor activity monitored and recorded at 24 hr.

A second series of experiments was designed to test increased doses (50-200 nmol, dynorphin A 1-13, i.t.) in adult Sprague Dawley rats (N = 22). The same stimulation and recording procedures were used throughout. The motor scores of the animals were recorded at 24 hr. Motor scores were assigned using the following criteria in both experiments:

Score	Function
3	Normal motor function
2	Paraparesis, with ability to support weight and walk with impairment, or make walking movements without supporting weight
1	Severe paraparesis, in which animals could make voluntary hindlimb movements but not walking movements
0	Total paralysis, with complete absence of any hindlimb movement

Results

Neuroanatomical evaluation consistently revealed extensive neural injury throughout the sacral cord and lumbar enlargement following injection of 20 nmol of dynorphin A (1-17). The neuropathological changes, found throughout the gray matter, were characterized by wide-

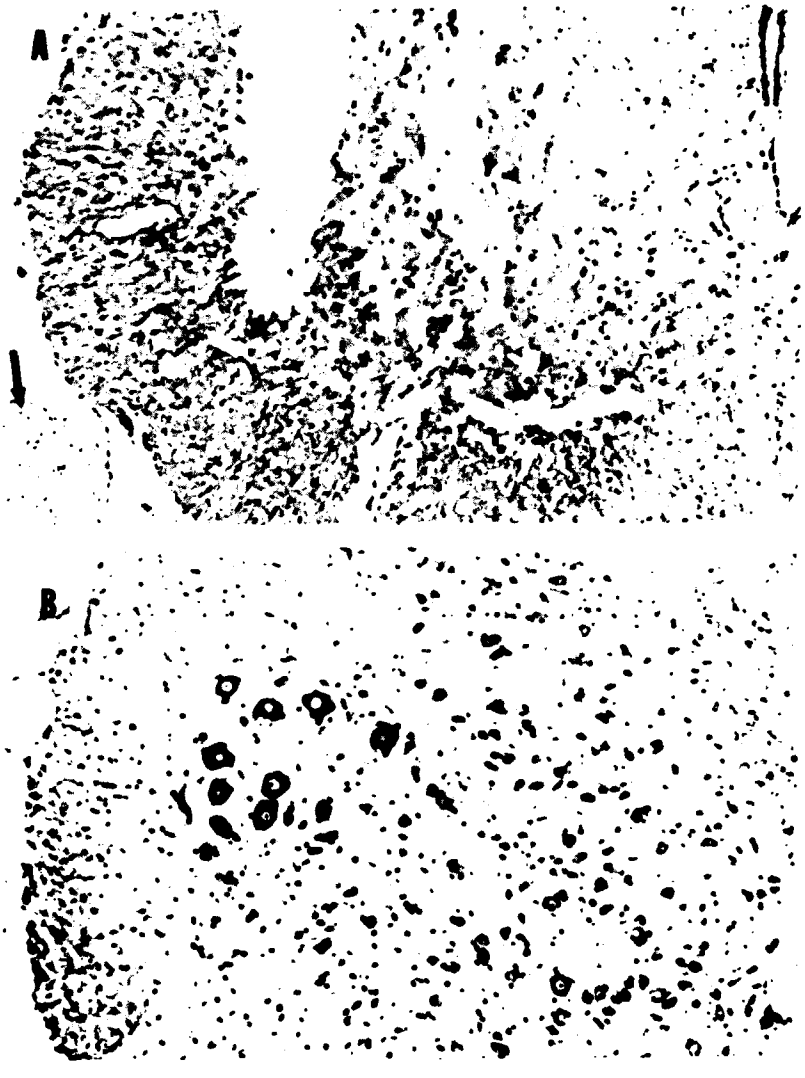

Fig. 1. Light photomicrographs of the lower lumbar (L6) spinal cord perfused 72 hr after injection (i.t.) of either dynorphin (A) or dynorphin with ketamine (2 μmol, B). Note extensive tissue necrosis following peptide exposure. (A) These paralyzed animals show extensive neuropathological changes in the gray and white matter with both neurons and glia being involved. Segmental ventral roots (arrow) are also affected. This is typical of the injury seen throughout the lumbosacral cord. However, dynorphin injected rats cotreated with ketamine (B) maintain neurons in the ventral horn with glial proliferation and only slight changes in the white matter of the sixth lumbar spinal cord segment. Nissl stain. X25.

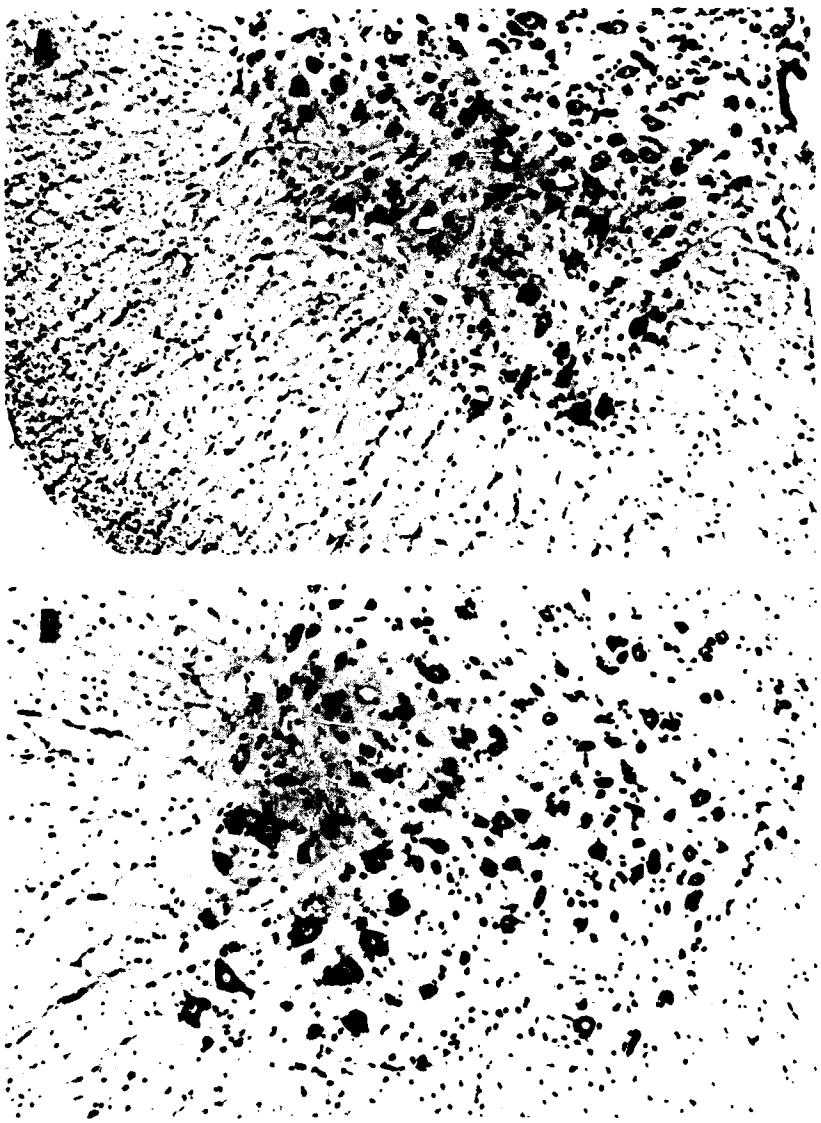

Fig. 2. Light photomicrographs of the lumbar enlargement from a dynorphin plus ketamine (4 μmol, A) and a saline injected control (B). The ketamine treated animals show some loss of motoneurons and slight glial proliferation when compared to control animals at 72 hr postinjection. Nissl stain. A, X25; B, X50.

spread ischemic and necrotic cell changes, diffuse gliosis, and cellular infiltration in these paralyzed animals (Fig. 1A). In contrast, after coinjection of dynorphin and ketamine, animals showed sparing of dorsal and ventral horn neurons in the lower lumbar segment (L6) and lumbar enlargement (Figs. 1B, 2). Mean histopathological grades for each treatment group are shown in Figs. 3, 4, and 5. Dynorphin treated animals were uniformly paralyzed and exhibited extensive neuropathology throughout the lumbosacral cord. Rats injected with dynorphin plus ketamine (2 mol) exhibited protection of the lumbar enlargement and lower lumbar (L6) segment. Rats treated with dynorphin plus ketamine (4 μmol) exhibited a greater degree of protection at the same levels of the spinal cord (lumbar enlargement and L6). All animals treated with either dose of ketamine plus dynorphin were weight bearing.

The neurophysiological responses (SSEP) in preliminary experi-

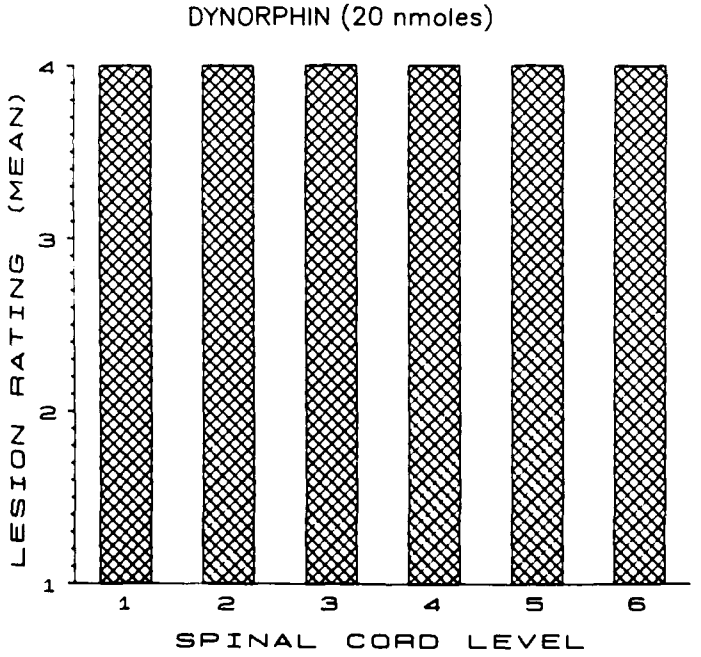

Fig. 3. Summary of histopathological results following dynorphin plus saline intrathecal coinjection to the lumbosacral cord in halothane anesthetized animals (N = 4). Transverse (1,3,5) and horizontal (2,4,6) sections were evaluated by 3 observers and graded on a scale of 1-4. Dynorphin (20 nmol) produced extensive neuropathological changes (grade 4) in all spinal cord segments examined in these paralyzed animals 72 hr postinjection.

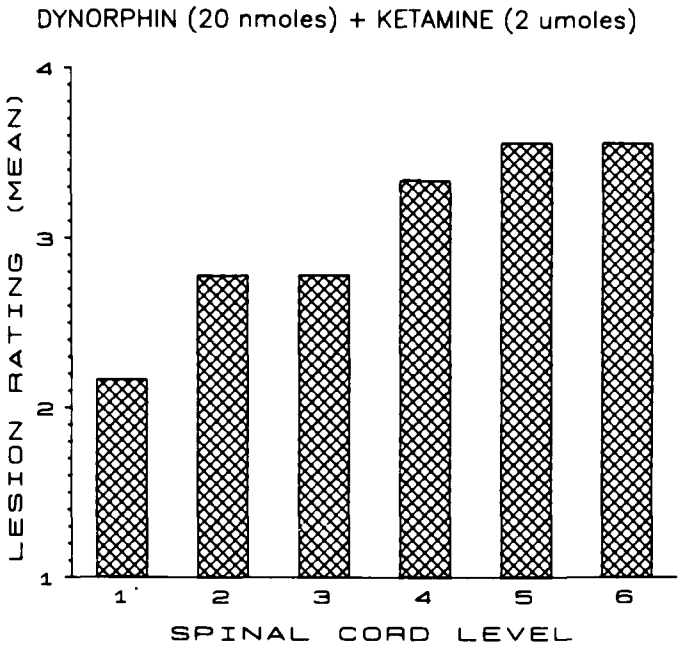

Fig. 4. Summary of histopathological results following dynorphin and ketamine (2 μmol, N = 4) intrathecal coinjection. Maximum protection was observed in the lumbar enlargement (level 1,2) and the sixth lumbar spinal cord segment (level 3). The sacral cord exhibited minimal protection in all of these animals which could support their own weight but walked with impairment.

ments with animals receiving 50 nmol of dynorphin while anesthetized with ketamine for several hours (2-4) showed little change in either latency or amplitude of the cortical response (Fig. 6). The subsequent motor scores were rarely abnormal. However, increased doses of dynorphin (100-200 nmol) in ketamine anesthetized rats produced a reduction of the SSEP beginning shortly after the injection of dynorphin (Fig. 7). In these animals, there was a correlation between the length of time of SSEP reduction with the resultant motor score (Fig. 8). Specifically, all animals showing a 50% reduction in the maximum amplitude of the SSEP of <30 min were walking and weight bearing at 24 hr and all but one of the remaining animals were either paralyzed or expired.

Discussion

When injected intrathecally, dynorphin produces a significant dose related reduction in blood flow in the rodent lumbosacral cord (37-75%)

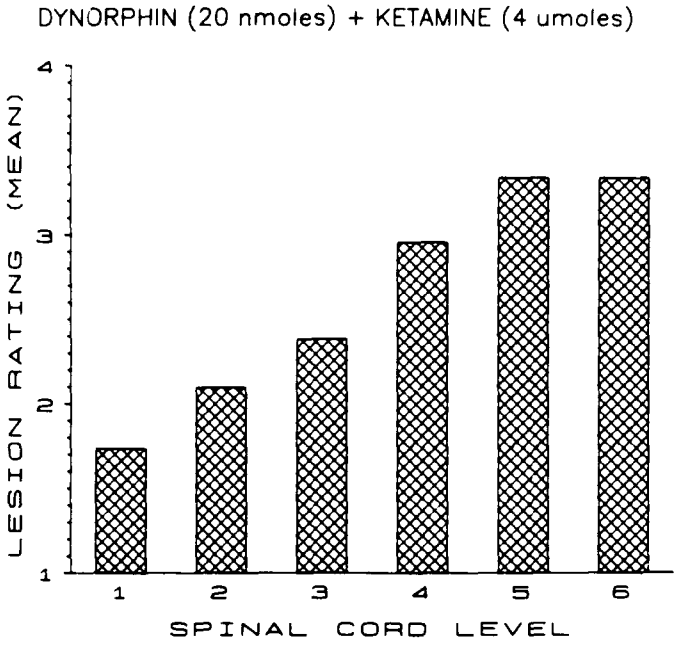

Fig. 5. Summary of histopathological results following dynorphin and ketamine (4 μmol, N = 7) intrathecal coinjection. Maximum protection was observed in the lumbar enlargement and was dose dependent. All animals could walk and were weight bearing.

without altering cardiac output or blood flow to the brain or cervical spinal cord (Long *et al.*, 1987). The resultant secondary changes in nociceptive responses, loss of EMG activity, and paralysis are thought to be caused, at least in part, by this ischemic insult. Neuropathological changes found after dynorphin exposure parallel the ischemic spinal cord changes reported to occur in a variety of animals following aortic occlusion (DeGirolami and Zivin, 1982). This injury primarily produces central gray matter necrosis and glial proliferation. It secondarily causes edema in the surrounding white matter. Interestingly, both ketamine and MK-801 (Rigamonti *et al.*, 1989), two NMDA receptor antagonists, attenuate these neuropathological changes. Both intra- and extracranial blood vessels of the rat are innervated by neural fibers containing dynorphin, and presumably receptors located on the underlying blood vessels respond to their input (Moskowitz *et al.*, 1987). We have observed that the vasoconstriction of pial vessels of the parietal cortex by topical

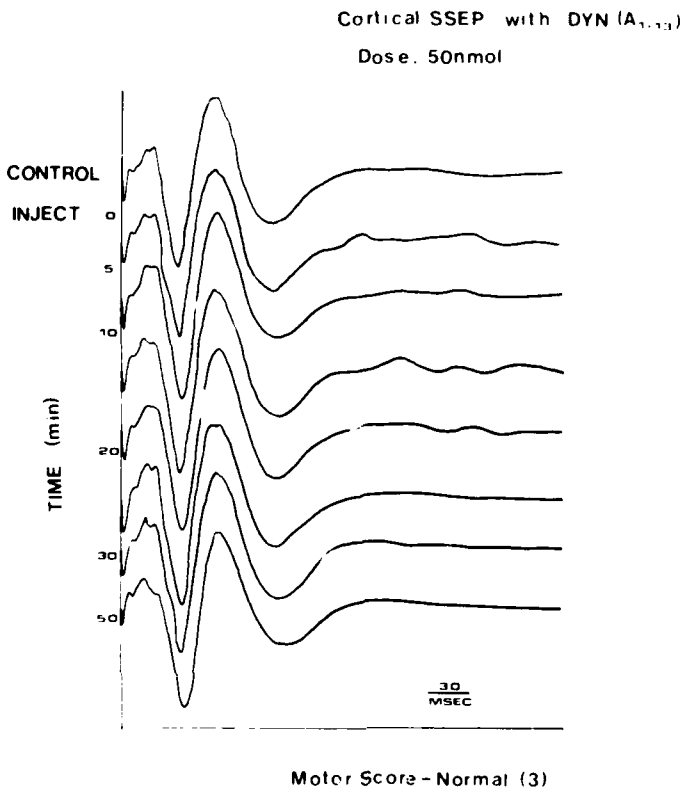

Fig. 6. Somatosensory evoked potential (SSEP) recorded from the cerebral cortex in the ketamine anesthetized animal following stimulation of the contralateral sciatic nerve. Following injection of 50 nmol of dynorphin, a dose which always produced a loss of the SSEP in halothane anesthetized animals, the SSEP amplitude and latency were unaffected for 50 min. The motor score at 24 hr was normal.

dynorphin application appears to be attenuated by simultaneous exposure to ketamine (unpublished observations). Thus: 1) vascular receptors for dynorphin associated with similar innervations in the spinal cord may account for the blood flow reductions and the resultant ischemic response to dynorphin exposure, and 2) ketamine may blunt dynorphin induced vasoconstriction and thereby improve recovery.

The cortical somatosensory response (SSEP) decreases following a reduction of spinal cord blood flow (Berenstein *et al.*, 1983; Schramm and Jones, 1985), and changes in the SSEP 5-10 min after dynorphin

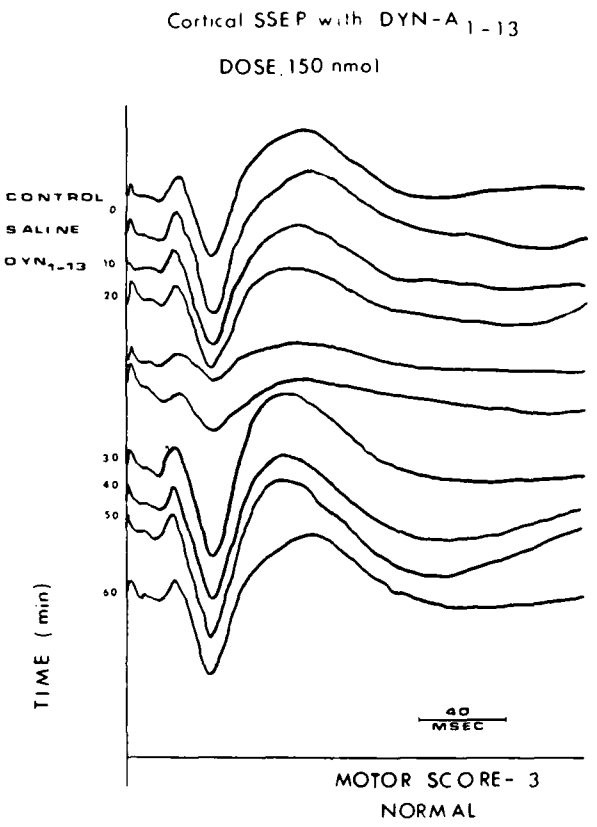

Fig. 7. Cortical SSEP recorded in ketamine anesthetized rats following contralateral sciatic nerve stimulation. There was a decreased amplitude for only 10 min following injection of 150 nmol of dynorphin and normal motor function 24 hr postinjection. This dose always produced permanent paralysis in halothane anesthetized animals and persistent loss of the SSEP.

exposure correlate well with other reported effects of ischemia on long tract neural conduction in the spinal cord (Kobrine *et al.*, 1979). The loss of the cortical response for greater than 30 min has also been used to predict paralysis in the clinical setting following spinal cord surgical procedures. The loss of the SSEP for greater than 30 min was a reliable indicator to predict paralysis in this model. Of current interest are changes not only in the dorsal column, the location of fibers which transmit the SSEP to the cerebral cortex, but also in segmental reflex activity in the spinal cord following dynorphin exposure. Spinal cord reflexes are altered when exposed to dynorphin (Caudle and Isaac, 1988) and ketamine

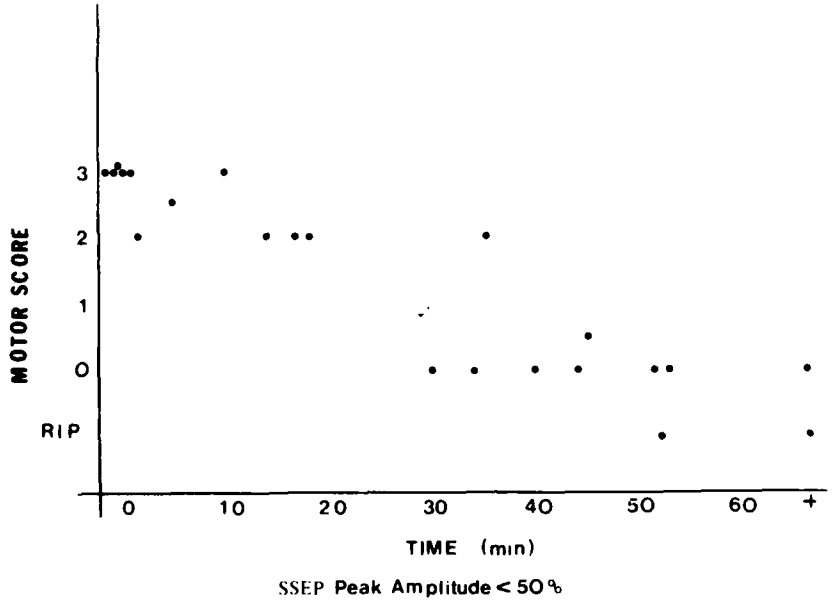

Fig. 8. Comparison of change in the somatosensory evoked response (SSEP) and subsequent motor score of animals (N = 22) receiving dynorphin (i.t. 50-200 nmol) while anesthetized with ketamine After recording a baseline cortical SSEP (control) the lumbosacral cord was exposed to dynorphin and the change in maximum peak amplitude over time was measured. All animals with a 50% reduction of the maximum peak amplitude of the SSEP for <30 min were weight bearing the following day (Motor score 3 is normal and 0 is paralyzed). Animals (88%) with a 50% reduction of the maximum peak response for greater than 30 min were subsequently paralyzed or expired (RIP). All animals receiving 50-200 nmol of dynorphin with halothane anesthesia were consistently paralyzed.

(Lodge and Anis, 1984). We are currently examining the interaction of ketamine and dynorphin when simultaneously applied to the spinal cord.

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statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH Publication 86-23, 1985 edition."

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