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Topical application of dynorphin A (1–17) antiserum attenuates trauma induced alterations in spinal cord evoked potentials, microvascular permeability disturbances, edema formation and cell injury

An experimental study in the rat using electrophysiological and morphological approaches

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Summary. Dynorphin is a neuropeptide that is present in high quantities in the dorsal horn of the spinal cord. The peptide is actively involved in pain processing pathways. However, its involvement in spinal cord injury is not well known. Alteration in dynorphin immunoreactivity occurs following a focal trauma to the rat spinal cord. Infusion of dynorphin into the intrathecal space of the cord results in ischemia, cell damage and abnormal motor function. Antibodies to dynorphin when injected into the intrathecal space of the spinal cord following trauma improve motor recovery, reduce edema and cell changes. However, influence of dynorphin on trauma induced alteration in spinal cord bioelectrical activity is still not known. Spinal cord evoked potentials (SCEP) are good indicator of spinal cord pathology following trauma. Therefore, in present investigation, influence of dynorphin antibodies on trauma induced changes in SCEP were examined in our rat model. In addition, spinal cord edema formation, microvascular permeability disturbances and cell injury were also investigated. Our results show that topical application of dynorphin antiserum (1 : 200) two min before injury markedly attenuated the SCEP changes immediately after injury. In the antiserum treated animals, a significant reduction in the microvascular permeability, edema formation and cell injury was observed in the traumatised spinal cord. These observations suggest that (i) dynorphin is involved in the altered bioelectrical activity of the spinal cord following trauma, (ii) the peptide actively participates in the pathophysiological processes of cell injury in the spinal cord trauma, and (iii) the dynorphin antiserum has potential therapeutic value for the treatment of spinal cord injuries.

Keywords: Amino acids – Dynorphin antiserum – Spinal cord injury – Edema – Spinal cord evoked potentials – Ultrastructure

Introduction

The involvement of neuropeptide dynorphin in the pathophysiology of spinal cord injury is not well characterised. Dynorphin A is an endogenous opioid neuropeptide and the major product of the preprodynorphin gene (Civelli et al., 1985). The dynorphin is mainly regarded as an inhibitory neurotransmitter involved in the central pain pathways. Exposure of the spinal cord to a high concentration of dynorphin will induce hyperalgesia and allodynia (Evans et al., 1988). However, recent evidences suggest that the peptide may also be involved in the central mechanisms of neurodegeneration (Faden, 1993, 1996; Sharma et al., 1995). This is indicated by the fact that dynorphin A and related peptides are significantly elevated in brain and spinal cord following injury (Faden 1993; Sharma et al., 1992; 1995). Moreover, intrathecal infusion of the peptide induces motor dysfunction and hind limb paralysis (Faden 1990). In these animals widespread ischemia and neurotoxicity in the spinal cord are common findings (Faden 1990; cf Sharma et al., 1995).

Dynorphin fibers are densely distributed in lamina I and II throughout the whole spinal cord (Hökfelt et al., 1978). Dynorphin fibres are also scattered in

laminae IV–VII, the dorsal gray commissure, lamina X, intermediolateral cell column and intermedial cell cluster (Björklund et al., 1990; Fallon and Ciofi 1990). Immunocytochemical studies combined with axonal degeneration at the ultrastructural level suggest that dynorphin immunoreactive cell bodies in lamina I receive direct primary and non primary afferent input from small-caliber fibers (Cho and Basbaum, 1989). In this layer, dynorphin and enkephalins are co-localised (Hökfelt et al., 1978; Fallon and Ciofi, 1990). This observation suggests a direct role of opioid containing cells in the transmission of nociceptive inputs through the dorsal horn. A direct role of dynorphin containing cells in the dorsal horn in nociceptive input is further suggested by the studies using chronic stress, arthritis, peripheral inflammation and deafferentation (Smith and Lee, 1988). In these conditions, an upregulation of dynorphin occurs in the lamina I of the dorsal horn (see Björklund et al., 1990; Fallon and Ciofi, 1990). Following hyperalgesia, a 300% increase in dynorphin occurs in the spinal cord dorsal horn (Iadarola et al., 1988). These observations indicate that dynorphin system in the spinal cord is sensitive to peripheral alterations in the noxious input.

There are evidences that the major product of dynorphin A (Dyn A 1–13 and Dyn A 1–17) will cause neuronal injury probably through a non-opioid mechanism (Chen et al., 1995). This is supported by the finding that the deleterious effects of the dynorphin A can be prevented by N-methyl-D-aspartate (NMDA)-glutamatergic receptor antagonist MK-801 but is unaffected by opioid antagonists (Isaac et al., 1990). In spinal nerve ligation injury models, antiserum to dynorphin A has the same profile of actions as the NMDA receptor antagonists MK-801 in blocking thermal hyperalgesia and restoring the efficacy of morphine against allodynia (Noichols et al., 1997). However, in spite of widespread distribution of dynorphin and its involvement in cell injury in the spinal cord, the role of peptide in the brain or spinal cord injury following trauma is still not well known.

Previous studies from our laboratory demonstrate that dynorphin A (1–17) content of the spinal cord was increased in the T9 segment following an incision into the right dorsal horn of the T10–11 segments (Sharma et al., 1992). In these animals, edema formation and cell injury are quite prominent in the T9 segment (Sharma and Olsson, 1990). These observations suggest that increased dynorphin level may somehow contribute to the cell injury in the spinal cord. This idea is further supported from the observations that topical application of dynorphin A (1–17) antiserum (1 : 20 dilution in the phosphate buffer) two min after the injury was able to significantly reduce the edema formation and cell injury in the cord (Sharma et al., 1995). This observation is in the line with the idea that dynorphin antiserum is neuroprotective in the spinal cord.

There are abundant experimental and clinical evidences that the spinal cord evoked potentials (SCEPs) are one of the important indicators of spinal cord pathology (Sharma et al., 1991; Stålberg et al., 1998). Experimental observations from our laboratory suggest that the SCEPs changes following trauma can be influenced by pretreatment with drugs known to influence spinal cord pathology (Winkler et al., 1998). Thus, pretreatment with neuroprotective drugs capable to reduce edema formation and cell damage in the cord is able to markedly attenuate the early SCEP changes seen immediately or within 2 to 4 min after trauma. On the other hand drugs that are unable to induce any beneficial effects on edema formation and cell injury did not influence early SCEP changes following injury (Sharma et al., 1991; Sharma and Winkler, 2002). Since, the dynorphin is mainly regarded as an inhibitory neurotransmitter involved in the pain pathways (Weisskopf et al., 1993; Fallon and Ciofi, 1990), it seems likely that the peptide can influence SCEP changes following spinal cord trauma as well. However, its role in spinal cord injury induced bioelectrical disturbances are not yet known (Nyberg et al., 1995). Thus, in present investigation, the influence of dynorphin A antiserum on trauma induced alterations in the SCEP was examined in our rat model. Furthermore, the microvascular permeability disturbances, edema formation and cell injury in the spinal cord were also examined in the antiserum treated rats.

Materials and methods

Animals

Experiments were carried out on 38 male Sprague-Dawley rats weighing between 400–460 g body weight. The animals were kept at controlled ambient temperature 21 ± 1°C with 12 h light and 12 h dark schedule. Standard laboratory rat food pellets and tap water were provided ad libitum before the experiment.

Spinal cord injury

Under Equithesin anaesthesia (3 ml/kg, i.p.) one segment laminectomy was done at the T10–11 level. Spinal cord injury was inflicted

by making a longitudinal incision into the right dorsal horn using a sterile scalpel blade under aseptic conditions. The wound was covered with cotton soaked in 0.9% saline to avoid the direct exposure of the cord to the air (Sharma and Olsson, 1990). Animals were allowed to survive for 5 h after injury. This experimental condition is approved by the Ethics Committee of Uppsala University, Uppsala, Sweden.

Control group

Normal animals under Equithesin anaesthesia were used as controls. We did not find any significant difference between control or sham operated animals regarding the changes in microvascular permeability, water content and morphology of the cord (Winkler et al., 1998). Thus all the comparisons between untreated injured animals or antiserum treated rats in this study were made in relation to normal intact rats.

Treatment with dynorphin A (1–17) antiserum

The commercial dynorphin A antiserum (1–17, Calbiochem, USA, polyclonal) was used in this investigation (dilution 1 : 200 in phosphate buffer saline, 0.1 M, pH 7.0). The Dynorphin A antiserum (20 μl in 10 sec) was applied over the exposed spinal cord 2 min before injury. These animals were allowed to survive 5 h after the injury (Sharma et al., 1995).

Recording of spinal cord evoked potentials

The SCEPs were recorded from the specially prepared epidural electrodes (Fig. 1) as described earlier (Sharma et al., 1991). In brief, a denuded copper wire (o.d. 0.1 mm) was inserted into the lumen of the polyethylene catheter PE 10 (2.5 cm) and its proximal end was taken out and wrapped around the tube to form an extended surface area of the recording electrode over the spinal cord (Sharma et al., 1991; Winkler, 1994). The distal end of the electrode was connected to the EMG machine (Medtronic Functional Systems, Copenhagen). The SCEPs were recorded after a supramaximal electrical stimulation of the right tibial and sural nerves (for details see Sharma et al., 1991; Winkler et al., 1998).

Analysis of the SCEPs

The mean negative amplitudes (MNA), mean positive amplitude (MPA) and their latencies were used for calculating SCEP changes (Fig. 1) as descried earlier. The SCEP amplitudes and latencies were used as 100% before the injury. Comparisons were made with the SCEP recording in each animal obtained 30 min before (−30 min) injury (Sharma et al., 1991).

Blood-spinal cord barrier permeability

The blood-spinal cord barrier (BSCB) permeability was examined using Evans blue (2% in sterile saline solution, pH 7.4) and $^{[131]}$Iodine as protein tracers (Sharma, 1987). At the end of the experiment, under Equithesin (3 ml/kg, i.p.) anaesthesia the Evans blue (0.3 ml/100 g) and $^{[131]}$Iodine (10 μCi/rat) tracers were injected into the right femoral vein through a needle puncture. The tracers were allowed to circulate for 5 min. The intravascular tracers were washed-out with 0.9% saline perfusion through heart followed by perfusion with a paraformaldehyde based fixative (Sharma et al., 1990, see below). After perfusion, the spinal cord was removed and examined for Evans blue extravasation. After that, the spinal cord was divided into the T9 and T10–11 segments, weighed immediately and counted in a 3-in well type Gamma counter (Packard, USA; energy window 50–80 KeV). A sample of the whole blood was withdrawn through cardiac puncture to measure the whole blood radioactivity immediately before perfusion. Extravasation of the radiotracer was expressed as percentage increase in the radioactivity in the cord over the blood radioactivity (Sharma, 1987). The Evans blue dye entered into the cord was measured colorimetrically as described earlier (Sharma, 1999).

Spinal cord edema

Spinal cord edema was determined using water content of the traumatised spinal cord according to differences in the wet and dry weights of the sample (Sharma and Olsson, 1990). In brief, spinal cord was removed immediately and weighed on the pre-weighed filter papers. The spinal cord samples were dried in an oven maintained at 90°C for 72 h or unless the last three dry weights of the cord became constant (Sharma and Olsson, 1990).

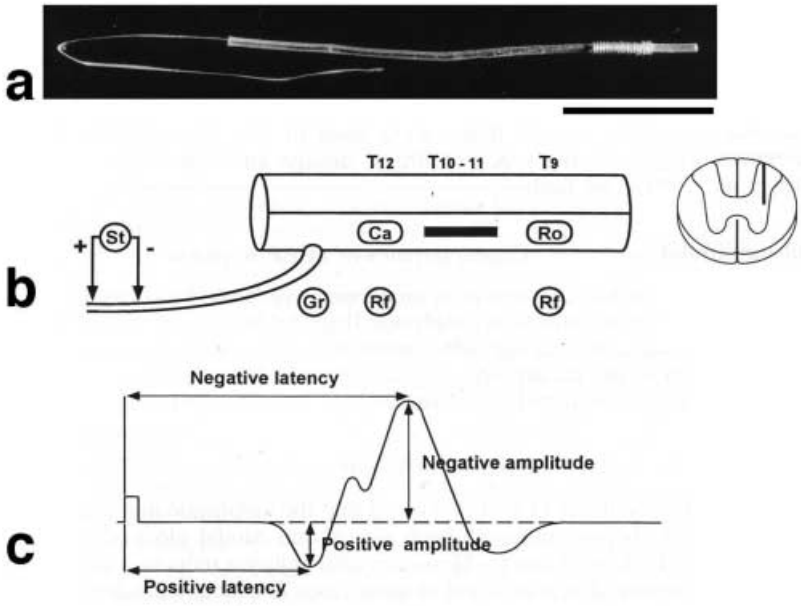

Fig. 1. Shows electrode, model and methods of SCEP recordings in the rat. **a** One epidural electrode (bar = 5 mm), **b** (*left*) Diagrammatic representation of spinal cord stimulation (*St*), placement of electrodes (gr = ground; *Rf*, reference; *Ro*, rostral, *Ca*, caudal) and lesion site (horizontal bar = 4 mm). **b** (*right*) Cross section of the spinal cord showing extent of the lesion of the dorsal horn (vertical bar = 2 mm). **c** Methods used for measurement of SCEP amplitudes and latencies

Morphology of the spinal cord

In order to examine the influence of dynorphin antiserum at the ultrastructural level, animals were perfused in situ with a formaldehyde based solution (2.5% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium-potassium phosphate buffer, pH 7.0 containing 2.5% picric acid) preceded with a brief saline rinse (Sharma et al., 1990). After perfusion, the spinal cord tissue pieces were removed and kept in the same fixative for one week. The tissue pieces from the T9 and T12 segments were processed for standard transmission electron microscopy. The ultrathin sections were contrasted with uranyl acetate and lead citrate and viewed under a Phillips or Hitachi electron microscope (Sharma and Olsson, 1990; Sharma et al., 1995; Sharma, 1999).

Statistical evaluation

The statistical significance between control, injured and antiserum-treated groups were analysed using ANOVA followed by Dunnet test for multiple group comparison. A p-value less than 0.05 was considered to be significant.

Results

Effect of dynorphin A antiserum on physiological variables

Spinal cord injury induced a mild but significant hypotension at 5 h compared to the control group (Table 1). The arterial pH and blood gases were not significantly different from the control group. Pretreatment with dynorphin A antiserum in the control group or the spinal cord traumatised animals did not influence these parameters significantly (Table 1).

Effect of dynorphin A antiserum on SCEP

The SCEP negative amplitude consisted of a broad and high negative amplitude before trauma (Fig. 2). In untreated rats, a focal trauma to the spinal cord resulted in an immediate depression of the SCEP negative amplitude (mean depression 40 ± 10%, range 60 to 100%) that lasted for 1 h (Fig. 3). At the same time, the SCEP positive amplitude appeared which continued to increase over 1 h period (Fig. 2). At 2 h period,

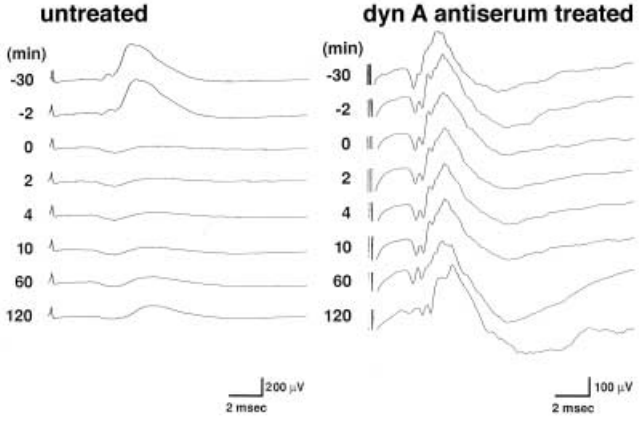

Fig. 2. Representative examples of SCEP recordings (0 to 120 min) in one untreated spinal cord injured rat and their modification with dynorphin A (1–17) antiserum. The SCEPs changes are most marked in the untreated rat compared to the dynorphin antiserum treated rat after injury (0 min)

Table 1. Physiological variables, spinal cord edema and blood-spinal cord barrier permeability in 5 h spinal cord traumatised rats and their modification with dynorphin A (1–17) antiserum

Dynorphin antiserum was administered topically (1 : 200 in phosphate buffer saline, pH 7.0 at room temperature) 2 min before injury (for details see text)

Parameters measured	Control	Dyn A antiserum	5 h SCI	Dyn A antiserum +SCI
A. Physiological Variables	n = 6	n = 5	n = 6	n = 6
MABP torr	118 ± 10	108 ± 9	82 ± 10**	86 ± 8**
Arterial pH	7.37 ± 0.08	7.36 ± 0.06	7.35 ± 0.09	7.36 ± 0.06
PaO_2 torr	81.89 ± 0.32	82.34 ± 1.04	79.35 ± 0.43	80.19 ± 0.45
$PaCO_2$ torr	34.67 ± 0.54	33.89 ± 0.65	33.34 ± 0.33	33.76 ± 0.28
B. Blood-spinal cord barrier permeability T9 segment	n = 5	n = 5	n = 8	n = 6
Evans blue mg %	0.24 ± 0.06	0.28 ± 0.06	1.58 ± 0.34**	0.86 ± 0.23**a
$^{[131]}$Iodine %	0.38 ± 0.08	0.40 ± 0.06	2.34 ± 0.54**	1.05 ± 0.33**a
C. Spinal cord water content %	n = 6	n = 5	n = 6	n = 6
T9	65.34 ± 0.12	65.23 ± 0.08	68.34 ± 0.45**	66.33 ± 0.28**a
T10–11	66.23 ± 0.18	66.01 ± 0.32	68.89 ± 0.48**	66.48 ± 0.43**a

Values are mean ± SD; *MABP*, mean arterial blood pressure; *SCI*, spinal cord injury; **P < 0.01 (compared from control group), *a*, P < 0.05 (compared from 5 h SCI group), ANOVA followed by Dunnet test for multiple group comparison

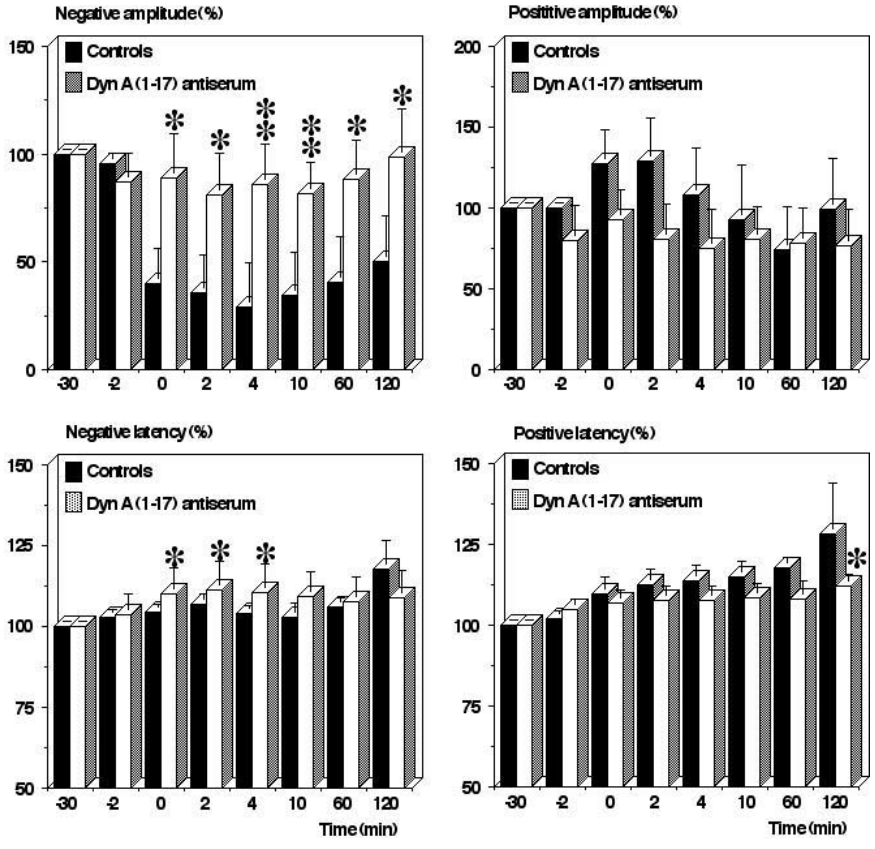

Fig. 3. Mean SCEP amplitudes (upper panel) and latencies (lower panel) in the untreated traumatised rats and their modification with dynorphin A (1–17) antiserum. The SCEP negative amplitude and latency exhibited significant increase after trauma in the dynorphin antiserum treated animals compared to the untreated traumatised group. *$P < 0.05$, ANOVA followed by Dunnet's test for multiple group comparison

appearance of a small SCEP negative peak was observed. However, the SCEP negative amplitude continues to remain significantly depressed compared to the pre-injury level throughout the period (120 min) of recording.

Pretreatment with dynorphin A antiserum did not significantly alter the shape of the SCEP negative amplitude before injury (Fig. 2). Interestingly, a focal trauma to the spinal cord in these antiserum-treated rats did not result in any diminution of the SCEP negative amplitude (Fig. 2). In fact, the SCEP negative amplitudes were increased from the pre-injury level after 120 min, although this increase in SCEP negative amplitude was not statistically significant (Fig. 3). In these antiserum treated and traumatised rats, the SCEP positive amplitude did not develop (Figs. 2 and 3).

However, we observed a significant increase in SCEP negative latencies following dynorphin A treatment. This increase in SCEP latency was significantly higher than untreated group at 0, 2 and 4 min after injury (Figs. 2 and 3).

Effect of dynorphin A antiserum on blood-spinal cord barrier permeability

Trauma to the spinal cord induced a marked extravasation of the Evans blue and radiotracer into the spinal cord T9 segment at 5 h compared to the controls (Table 1). There was a significantly higher permeation of the radiotracer in the spinal cord following trauma compared to that of Evans blue extravasation (Table 1). Dynorphin A antiserum treatment alone did not result in any significant differences in the leakage of these tracers into the spinal cord compared to the control group (Table 1). However, pretreatment with dynorphin A antiserum significantly attenuated the trauma induced extravasation of Evans blue and $^{[131]}$Iodine tracers at 5 h in the spinal cord. The magnitude of reduction in the tracer extravasation was most marked for Evans blue dye compared to the radioactive iodine.

Effect of dynorphin A antiserum on edema formation

A focal spinal cord injury induced profound edema formation in the traumatised cord (Table 1). This is

evident from the results obtained with measurement of water content of the spinal cord. Thus, there was a significant increase in the water content of the cord in the T9, and T10–11 segments following 5 h after trauma compared to the control group (Table 1). Pretreatment with dynorphin A antiserum significantly attenuated the trauma induced edema formation in these segments at 5 h. Interestingly, dynorphin A antiserum treatment alone did not alter the spinal cord water content compared to the control group (Table 1).

Effect of dynorphin A antiserum on ultrastructure of the cord

Ultrastructural studies revealed profound morphological alterations in the spinal cord dorsal and ventral horns after trauma. Thus, perivascular edema, vacuolation, membrane damage, nerve cell distortion, glial cell swelling and myelin vesiculation were common findings. These changes were most marked in the vicinity of the lesion site. Thus ipsilateral dorsal horn of the T9 and T12 segments showed most pronounced cell injury compared to the contralateral side in these segments. One example of ultrastructural changes in the right dorsal horn of the T9 segment is shown in Fig. 4a. Profound edema, vacuolation and myelin damage are evident in this untreated traumatised rat. Pretreatment with dynorphin A antiserum markedly attenuated the trauma induced structural changes in the spinal cord. Thus, myelin vesiculation, edema, vacuolation and membrane disruption are much less frequent in this antiserum treated traumatised rat (Fig. 4b). The effect of dynorphin antiserum was most marked in the contralateral the T9 and the T12 segments of the cord. Treatment of spinal cord with Dynorphin A antiserum alone did not show any morphological alterations in the cord compared to the intact control groups (results not shown).

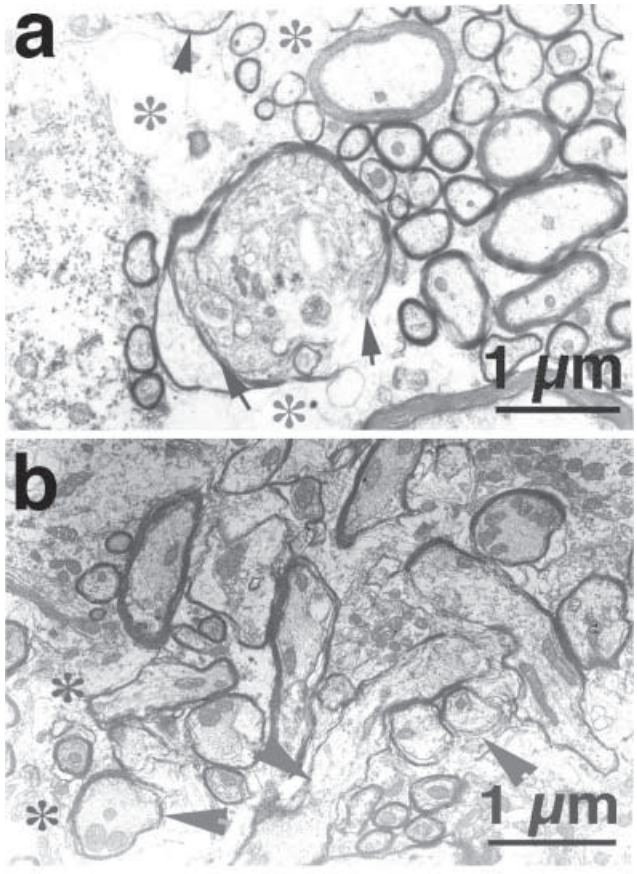

Fig. 4. Low power electron micrograph of the right dorsal horn of the T9 segment in one untreated 5 h injured rat (**a**) and its modification with dynorphin A antiserum (**b**). Myelin vesiculation (arrows) and membrane disruptions (*) are quite common in the untreated injured rat (**a**). In the dynorphin A antiserum treated rat, myelin vesiculation and membrane disruption after trauma are much less common

Discussion

The salient new findings of the present investigation show that the topical application of dynorphin A antiserum before spinal cord injury has the capacity to attenuate trauma induced changes in SCEPs. This observation suggests that the dynorphin peptide is somehow contributing to the SCEP changes, not reported earlier. These observations are the first to show that the SCEPs are largely preserved in the dynorphin A antiserumtreated and traumatised rats. This finding may have clinical application in the near future. Thus, it would be interesting to see whether application of dynorphin A antiserum applied several time periods, e.g., 10 min, 30 min or 60 min after trauma, is still beneficial in attenuating SCEP changes. However, this is a feature that requires additional investigation.

The mechanisms by which dynorphin A antiserum attenuated the SCEP changes are not clear from this investigation. There are evidences that dynorphin modulates synaptic transmission in the CNS and acts as a neurotransmitter (Weisskopf et al., 1993). Dynorphin affects the activity of individual neurons in many regions of the CNS. Both excitatory and inhibitory effects of dynorphin (1–17) on spontaneous and evoked activity have been described (North, 1993). The excitatory effects of dynorphin are usually antago-

nised by naloxone, whereas the inhibitory effects are not affected (Duggan and Fleetwood-Walker, 1993). The electrophysiological effects of dynorphin are blocked by naloxone, suggesting the involvement of multiple opioid receptors. The selective κ-opioid receptor antagonist nor-binaltrophimine (nor-BNI), completely blocks the inhibitory effects of dynorphin in a concentration of the drug which has no effect on μ or δ-opioid receptors (Smith and Lee, 1988).

This idea is further supported by our previous findings that high doses of naloxone (10 mg/kg) is able to attenuate SCEP changes following spinal cord injury (Winkler et al., 1994). In this study, low doses of naloxone (1 and 5 mg) are not able to attenuate SCEP changes (Winkler et al., 1994, 1998). In fact, the SCEP changes were further deteriorated following trauma in the animals which received the 1 mg/kg dose of the compound (Winkler et al., 1994). These observations suggest that the high dose of naloxone is capable to antagonising κ-opioid receptor that is needed to have a beneficial effect on the SCEP changes following trauma. The dynorphins are the natural ligands of the κ-opioid receptors (Chavkin and Goldstein, 1981). Thus, it is quite likely that blockade of κ-opioid receptors either by naloxone or antiserum to dynorphin will attenuate SCEP changes following spinal cord injury.

The multiple opioid receptor antagonist naloxone is known to improve sensory motor function and ischemia in experimental spinal cord injuries (Faden, 1993; Winkler et al., 1994, 1998). However, it still remains unclear which opioid receptors are involved in cord pathology and how they exert their damaging effects in the CNS injuries. Interestingly, dynorphin fragments (2–17) and (3–13) which have no activities at opioid receptors are also capable to induce hind-limb weaknesses (Faden, 1990). These observations suggest that dynorphin induced paralysis is mediated via both opioid and non-opioid receptors mediated mechanisms.

In several *ex-vivo* electrophysiological studies employing hippocampal slices, the dynorphin acts mainly as an inhibitory neurotransmitter (Smith and Lee, 1988; Wiesskopf et al., 1993). This is in line with the idea that a release of the peptide following trauma will induce inhibition of the spinal cord bioelectrical activity. An immediate depression of the SCEPs negative amplitude following trauma supports this assumption. We observed almost no depression of the SCEPs negative amplitude following trauma in the dynorphin A antiserum treated animals. This observation suggests that the dynorphin antiserum was able to block the inhibitory effects of the dynorphin after trauma. The dynorphin A antiserum penetrates rapidly into the spinal cord and binds with *in vivo* dynorphin antigens (Han and Xie, 1984). As a result, the inhibitory effects of dynorphin on the bioelectrical events in the spinal cord are attenuated. Taken together, our results suggest that the opioid peptide dynorphin is actively involved in the bioelectrical disturbances in the cord following trauma.

Our results further show that pretreatment with dynorphin A antiserum also attenuated the microvascular permeability disturbances, edema formation and cell injury. This observation suggests that the dynorphin is also contributing to the spinal cord pathology. The concept that endogenous opioids are involved in the pathophysiological processes following spinal cord injury is mainly derived from the early studies using experimental shock and brain injury (Amir, 1982).The dynorphin levels in the CNS are altered in trauma, pain and chronic stress (Smith and Lee, 1988; Sharma et al., 1992). The magnitude of this reaction correlates well with the severity of injury (Faden, 1993; 1996). It is interesting to note, the dynorphin 1–17 but not enkephalins and β-endorphins accumulates at the lesion site following spinal trauma (Faden et al., 1987). This observation suggests that dynorphin A (1–17) is the active component of the peptide involved in the pathological processes of the spinal cord injury (Faden, 1990). A marked neuroprotective effects of dynorphin A antiserum (1–17) in our study further supports this hypothesis.

To further characterise the role of dynorphin in spinal cord tissue damage, several authors used intrathecal infusion of the peptide and examined the pathophysiological consequences within the spinal cord (Faden, 1993). Intrathecal administration of dynorphin produced paralysis of hind-limbs and tail, loss of nociceptive response, atonic paralysis of bladder and bowl, and loss of motor and autonomic reflexes (Long et al., 1989; Gaumann et al., 1990; Isaac et al., 1990). These neurologic deficits correlate well with the neuropathology changes of the spinal cord (Gaunmann et al., 1990; Long et al., 1994; Faden, 1996). Infusion of dynorphin A (1–17) and dynorphin A (1–13) causes dramatic reductions in the local blood flow, widespread ischemic cell damage, neuronal loss, necrosis, gliosis cavitation and vascular injury within 72 h (Long et al., 1988).

On the basis of on these observations Faden (1990) infused antiserum to dynorphin A (2–17) and showed neurological improvements in animals following spinal cord injury. Using specific opioid receptor antagonists and subtypes, Faden concluded that the beneficial effects of dynorphin A antiserum are related with both opioid and non-opioid mechanisms. Another study used application of dynorphin A (1–13) and a specific κ- agonist U50488H near laminae IV/V spino-cervical tract neurons of the cat (Fleetwood-Walker et al., 1988). The authors found a potent naloxone-sensitive inhibition of the both thermal and mechanical noxious stimuli from cutaneous origin, a feature not observed when these compounds are applied on substantia gelatinosa (Fleetwood-Walker et al., 1988). These observations suggest that the effect of dynorphin in the spinal cord is region specific and involves both opioid and non-opioid receptors mediated mechanisms.

The detailed mechanisms by which dynorphin can induce neurotoxicity are still speculative. There are evidences that dynorphin can stimulate glutamate release in the CNS by acting through N-methyl-D-aspartate (NMDA) receptors (Tang et al., 2000; Hauser et al., 2001). This idea gets further support with the findings that dynorphin antiserum has the capacity to antagonise NMDA receptors in vivo (Tang et al., 2000). The effects of dynorphin antiserum are far more potent in reducing allodynia induced pain than that of the classical NMDA receptor antagonist MK-801 (Nichols et al., 1997). This observation suggests that dynorphin can induce neurotoxicity through a NMDA receptor mediated mechanisms as well.

Works carried out in our laboratory further show that topical application of dynorphin A (1–17) antiserum markedly attenuated the changes in the BSCB permeability, edema and cell injury following spinal cord trauma in rats at 5 h (Sharma et al., 1995). The present study confirms our previous findings and further suggests that the antiserum even when applied 10 times more diluted (1 : 200) than earlier (1 : 20) is capable of attenuating spinal cord pathology. This observation suggests that a dose response study of dynorphin A antiserum on spinal cord pathophysiology is needed in the near future. There are reports that the dynorphin participates in inflammatory responses. Sydbom and Terenius (1986) reported an increased release of histamine from mast cells caused by dynorphin in a dose dependent manner. Chahl and Chahl (1986) found that dynorphin induces plasma extravasation. These observations are in good agreement with our study in hyperthermia. Thus it is likely that dynorphin may be responsible for breakdown of the BBB permeability either directly or through activation of other vasoactive agents, such as serotonin, prostaglandin and nitric oxide (Tang et al., 2000; Hauser et al., 2001; Sharma and Alm, 2001). Activation of these endogenous agents will obviously induce a breakdown of the BSCB permeability leading to the spinal cord edema formation and cell injury.

In conclusion, our results show that the peptide dynorphin is involved in the altered bioelectrical activity of the spinal cord following trauma and actively participates in the pathophysiological processes of cell injury in the spinal cord. Furthermore, the dynorphin antiserum may have potential therapeutic values for the treatment of spinal cord injuries in the future.

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