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Spinal cord evoked potentials and edema in the pathophysiology of rat spinal cord injury

Involvement of nitric oxide

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Summary. The possibility that nitric oxide is somehow involved in the early bioelectrical disturbances following spinal cord injury in relation to the later pathophysiology of the spinal cord was examined in a rat model of spinal cord trauma. A focal trauma to the rat spinal cord was produced by an incision of the right dorsal horn of the T 10-11 segments under urethane anaesthesia. The spinal cord evoked potentials (SCEP) were recorded using epidural electrodes placed over the T9 and T12 segments of the cord following supramaximal stimulation of the right tibial and sural nerves in the hind leg. Trauma to the spinal cord significantly attenuated the SCEP amplitude (about 60%) immediately after injury which persisted up to 1h. However, a significant increase in SCEP latency was seen at the end of 5h after trauma. These spinal cord segments exhibited profound upregulation of neuronal nitric oxide synthase (NOS) immunoreactivity, and the development of edema and cell injury. Pretreatment with a serotonin synthesis inhibitor drug p-chlorophenylalanine (p-CPA) or an anxiolytic drug diazepam significantly attenuated the decrease in SCEP amplitude, upregulation of NOS, edema and cell injury. On the other hand, no significant reduction in SCEP amplitude, NOS immunolabelling, edema or cell changes were seen after injury in rats pretreated with L-NAME. These observations suggest that nitric oxide is somehow involved in the early disturbances of SCEP and contribute to the later pathophysiology of spinal cord injury.

Keywords: Nitric oxide – Spinal cord evoked potentials – Edema – Cell changes – p-CPA – Diazepam – Immunohistochemistry

Introduction

Spinal cord injury is a serious clinical problem associated with profound neurological disability. The magnitude of such disability depends on the severity of the primary insult. Following primary injury, a series of secondary injury cascade will lead to the edema formation, cell injury and spinal cord pathology (Schwab and Bartholdi, 1996). Thus, efforts should be taken to minimise the consequences of secondary injury in order to reduce the neurological deficits of spinal cord injured victims.

One of the first indicator of spinal cord injury is a loss of spinal cord conduction which can be examined using several electrophysiological techniques (Stålberg et al., 1998). Thus recording of somatosensory evoked potentials, motor evoked potentials and spinal cord evoked potentials are used in the past to detect loss of conduction and damage to the spinal cord in both experimental and in clinical conditions (Winkler, 1994). Recent research on this aspect suggest that, out of these spinal bioelectrical activity, spinal cord evoked potentials (SCEP) seems to be a quite reliable indicator of spinal cord function (Tator, 1995). Experiments carried out in our laboratory in the past one decade are in line with the idea that SCEP can serve as a good indicator of later outcome of pathological changes and edema seen in the spinal cord (Sharma et al., 1991; Winkler, 1994). Thus, pharmacological pretreatment with compounds capable of attenuating edema and cell changes also reduced an early loss or disturbances in SCEP recordings (Winkler et al., 1993; 1994). On the other hand, compounds which did not prevent the SCEP alteration immediately after injury were those that failed to reduce the later development of edema formation and cell changes (Winkler et al., 1994; 1997b).

We have shown previously that serotonin, prostaglandins, opioids and histamine influence the early SCEP changes following spinal cord after injury and edema formation (Sharma et al., 1991; Winkler et al., 1993, 1994, 1995). However, *in vivo* situations, no single chemical compound or factor is involved in the pathophysiological reaction of cell injury following trauma. Thus, it appears that several neurochemicals or factors involved in neurotransmission or signal transduction mechanisms are contributing to the pathophysiological reaction of the spinal cord following trauma.

Recently, nitric oxide (NO) has emerged as one of the important free radical gases which influences neurotransmission and regulate signal transduction mechanisms in the CNS (Dawson and Dawson, 1996; Yamada et al., 1996). There are experimental evidences that NO is involved in the neurotoxicity caused by stroke, ischemia and infarction (Dawson and Dawson, 1996). Experiments done in our laboratory recently suggest that NO is also involved in the pathological mechanisms of edema formation following spinal cord injury (Sharma et al., 1996). However involvement of NO in spinal cord bioelectrical activity is still unknown.

The present investigation was carried out to examine the influence of NO in SCEP changes in the traumatised spinal cord using a pharmacological approach. We examined the influence of drugs on nitric oxide synthase (NOS)

immunostaining (Sharma et al., 1996) in the spinal cord following trauma in relation with the early SCEP changes in our rat model (Winkler, 1994).

Materials and methods

Animals

Experiments were carried out on male Sprague Dawley rats (250–350 g) housed at controlled ambient temperature (21 ± 1°C) with 12 h light and 12 h dark schedule. The rat feed and tap water were supplied ad libitum before the experiments.

Spinal cord evoked potentials

For this purpose, under equithesin anaesthesia (3 ml/kg, i.p.) one segment laminectomy (T10-11) was done to expose dorsal surface of the underlying spinal cord segment leaving dura matter intact (Sharma et al., 1991). The spinal cord evoked potentials (SCEP) was recorded using specially prepared epidural electrodes (Sharma et al., 1991) inserted into the epidural space through the exposed area of the spinal cord and advanced rostrally and caudally so that the exploratory electrodes are lying over dorsal surface of the right dorsal horn of the T9 and the T12 segments respectively. The reference needle electrodes were placed over the respective vertebral muscles near the active electrodes as described earlier (Sharma et al., 1991). For recording of SCEP right tibial and sural nerves were stimulated supramaximally and the response recorded and averaged using an EMG equipment (Keypoint, Dantec, Copenhagen) (for details see Winkler, 1994).

Spinal cord injury

Spinal cord injury was produced in laminectomised rats 30 min later by making an incision (about 2 mm deep and 5 mm long) into the right dorsal horn of T10-11 segments. The deepest part of the lesion was mainly located into the Rexed's lamina VII–VIII (Sharma et al., 1991). In these spinal cord injured rats the SCEP was recorded 2 min and immediately before injury (0 min) followed by 2 min, 5 min, 10 min, 30 min, 60 min, 120 min and 300 min after injury.

Pretreatment with drugs

In a separate group of rats following drugs were given before spinal cord injury.

p-Chlorophenylalanine (p-CPA, a serotonin synthesis inhibitor). This drug was administered – intraperitoneally (100 mg/kg/day) for 3 consecutive days. On the fourth day spinal cord injury was made. This dose and schedule of drug treatment effectively inhibits the serotonin synthesis in the CNS (Sharma et al., 1991).

Diazepam (anxiolytic drug). This drug was given once subcutaneoulsy (4 mg/kg) 30 min before laminectomy. This dose is sufficient to inhibit stress response in animals effectively for 4 h (Winkler et al., 1997a).

N_G-Nitro-L-arginine methyl ester (L-NAME, an inhibitor of nitric oxide synthase). This compound was administered intravenously (50 mg/kg) 30 min before laminectomy. There are reports that this dose of the compound effectively inhibits ischemia induced NO production in the brain (Dawson and Dawson, 1996).

Parameters measured

The following parameters were measured in controls, spinal cord injured and drug-treated spinal cord traumatised rats.

NOS immunohistochemistry. The NOS immunohistochemistry was measured in the T9 segment of the cord using free floating vibratome sections (60 μm thick) using standard protocol described by Sharma et al. (1996).

Edema of the spinal cord. The T10-11 segment of the spinal cord was used to determine edema of the spinal cord using water content (Sharma et al., 1991).

In all these drug treated rats SCEP was recorded 2 min and immediately before injury (0 min) followed by 2 min, 5 min, 10 min, 30 min, 60 min, 120 min and 300 min after injury as mentioned above.

Statistical analysis of the data

The quantitative data on SCEP changes were analysed using ANOVA followed by Dunnett test, whereas unpaired Student's t-test was applied for spinal cord edema and NOS immunohistochemistry measurement. A p-value less than 0.05 was considered significant.

Results

SCEP changes in spinal cord injury

Before injury, SCEP consists of a small positive deflection followed by a broad negative amplitude on both rostral and caudal recordings. A focal trauma to the rat spinal cord significantly attenuated the SCEP amplitude (about 60% mean values) immediately after injury on the rostral recordings which persisted up to 1 h (Fig. 1). There was some recovery on SCEP negative amplitude following 1 h and onwards which continued up to the recording period of 5 h. However, a significant increase in SCEP latency was seen at the end of 5 h after trauma.

NOS immunohistochemistry in spinal cord injury

A focal trauma to the rat spinal cord significantly increased the number of NOS positive nerve cells in the gray matter of the spinal cord. However the most significant increase in the NOS positive cells was found in the ipsilateral compared to the contralateral side of the cord (Fig. 2A).

Edema formation in spinal cord injury

Spinal cord edema measured in the traumatised rats showed about 4% increase in the water content (Fig. 2B) which is comparable to about 16% increase in volume swelling (Sharma et al., 1991).

Effects of drug treatment

Pretreatment with p-CPA or diazepam significantly attenuated the trauma induced decrease in SCEP amplitude (Fig. 1b, c). These drugs also significantly attenuated the NOS upregulation seen 5 h after trauma (Fig. 2). On the other hand, L-NAME treatment did not inhibit either the decrease in early SCEP amplitude (Fig. 1d) or NOS upregulation after 5 h injury (Fig. 2). p-CPA and diazepam significantly attenuated edema formation (Fig. 2) whereas L-NAME pretreatment did not reduce the water content increase after trauma (Fig. 2).

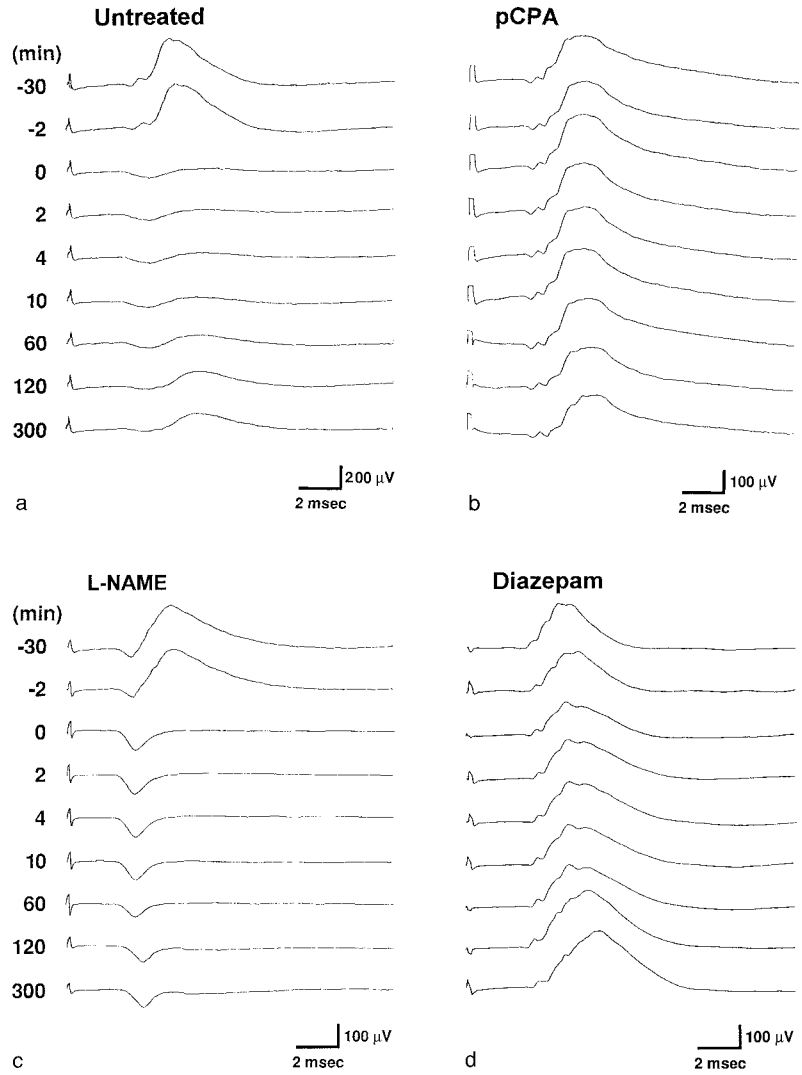

Fig. 1. Spinal cord evoked potentials recorded from epidural electrodes placed over T9 segment in one rat following spinal cord injury (**a**) and its modification with p-CPA (**b**), diazepam (**c**) and L-NAME (**d**). Note that L-NAME pretreatment did not prevent spinal cord injury induced changes in SCEP recordings

Discussion

The present results suggest that a focal spinal cord injury has the capacity to induce profound alterations in the SCEP activity recorded using epidural electrodes placed over the rostral and caudal segments of the spinal cord around a focal lesion. The most prominent sign of spinal cord dysfunction can be seen as the decrease of mean negative amplitude of SCEP. The other aspect of SCEP recording should be considered important is the increase in latency which occurred gradually throughout the period of 5 h.

It appears that trauma induced cascade of secondary injury mechanisms are playing important role in spinal cord dysfunction which can be reflected

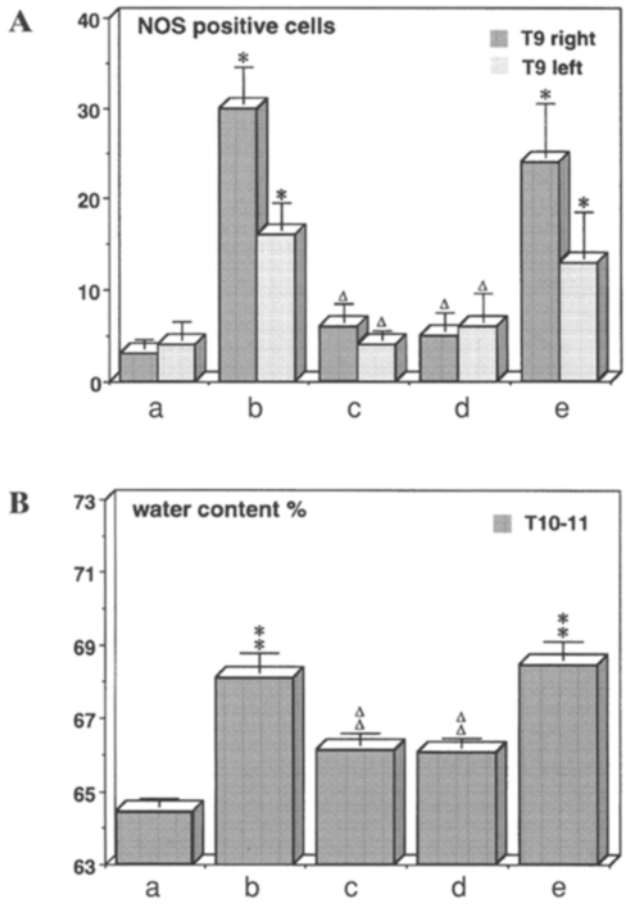

Fig. 2. **A** Semiquantitative data of NOS immunolabelled neurons in control, spinal cord traumatised and drug treated traumatised rats. *$p < 0.01$ compared from control, $\Delta = p < 0.05$, compared from spinal cord injured group. **B** Shows increase in water content in spinal cord traumatised rats and its modification with drugs. **$p < 0.01$, compared from control, $\Delta\Delta = p < 0.01$, compared from spinal cord injured group, Student's unpaired t-test. *a* control; *b* SCI; *c* pCPA + SCI; *d* diazepam + SCI; *e* L-NAME + SCI; *SCI* spinal cord injury

in SCEP changes (Tator, 1995; Schwab and Bartholdi, 1996; Stålberg et al., 1997). A lesion of the dorsal horn will induce a direct damage of nerve cells and their axons (Sharma et al., 1991). This will result in release of several neurotransmitters such as serotonin, prostaglandins, neuropeptides, histamine and many other neurochemicals (Winkler et al., 1997b). Most of the neurochemicals are localised in the superficial dorsal horn of the spinal cord gray matter. It is interesting to note that the enzyme nitric oxide synthase (NOS) is found co-localised in most of the dorsal horn neurons with other neuropeptides like substance P, CGRP and Neuropeptide Y for instance (Yamada et al., 1996). Apart from these neurotransmitters, enzymes responsible for prostaglandin, histamine and catecholamines are localised either on the microvessels or in nerve cells. It appears that the enzyme activity can also be influenced following trauma to the spinal cord (Schwab and Bartholdi, 1996).

A focal lesion of the dorsal horn will influence the release of several neurochemicals around the injury site. Most of the neurotransmitters released have the capacity to induce hyperpolarisation of the nerve cell membrane such as serotonin, prostaglandins and some neuropeptides. On the other hand, some neuropeptides can induce depolarisation of the membrane. Obviously, alterations in the spinal cord evoked potentials that we see after trauma represent an average of all these events. Apparently, pharmacological manipulation of neurochemical metabolism prior to trauma will influence the SCEP activity.

Another possibility regarding influence of neurochemicals on SCEP changes could be an indirect influence on "spinal shock" phase. The spinal shock will temporarily block conduction and/or nerve impulse for some time in the perifocal regions of the cord due to the severe cellular stress caused by trauma (Sharma et al., 1995). Since cellular stress can be influenced by a large number of neurochemicals, it seems quite likely that release of neurochemicals following a lesion of the spinal cord will also influence "spinal shock" phase (Sharma et al., 1995). Thus as a result, pharmacological manipulation of these neurochemicals may attenuate or amplify the spinal shock response resulting in alteration in spinal cord conductivity.

That neurochemicals may influence the early SCEP changes following trauma is evident from the present study, also reported earlier (Sharma et al., 1991; Winkler et al., 1997b). Thus, pretreatment with a serotonin synthesis inhibitor, p-CPA, or an antistress drug diazepam significantly inhibited the SCEP alterations after trauma. Since serotonin is a well known stress hormone, it appears that neurochemicals can influence the cellular stress response following trauma which can be reflected in early bioelectrical activity (Sharma et al., 1995). The ability of diazepam in reducing SCEP changes further emphasise the role of spinal shock as one of the important factors in SCEP alterations (Winkler et al., 1997a). Obviously modification of stress response by pharmacological compounds may have a direct influence on the consequences of secondary injury processes following trauma.

There are evidences that NO is involved in edema and cell injury following trauma (Sharma et al., 1996). However, the detailed mechanisms of NO production following trauma is not known. Since p-CPA and diazepam reduced the NOS upregulation following spinal cord injury in the present investigation, it appears that trauma induced alterations in neurochemical metabolism are involved in NO production. Alternatively NO production is somehow involved in the early disturbances of spinal cord conduction. The idea that increased NO production is involved in alterations in SCEP and spinal cord dysfunction is further supported by the results obtained with L-NAME pretreatment. L-NAME in the dose used here was neither able to inhibit NOS upregulation nor to attenuate the decrease in SCEP amplitude after trauma. In this group of rats profound edema and cell changes were evident at 5 h.

These observations suggest that NO contributes to the edema formation and cell injury following trauma to the spinal cord which can be well reflected in early SCEP changes. These results in general suggest that early SCEP

changes following trauma could be used as reliable indicator of later spinal cord dysfunction (for details see Stålberg, Sharma and Olsson, 1998; Winkler et al., 1997b). To our knowledge, this investigation is the first to demonstrate that NO is involved in the early bioelectrical disturbances following spinal cord injury. However additional studies using selective inhibitors of various isoforms of NOS are needed to further support this hypothesis.

In conclusion, our studies suggest that an upregulation of NOS following spinal cord injury is somehow related to SCEP alterations and spinal cord pathology.

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