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Growth hormone attenuates alterations in spinal cord evoked potentials and cell injury following trauma to the rat spinal cord

An experimental study using topical application of rat growth hormone

T. Winkler[1], **H. S. Sharma**[2], **E. Stålberg1**[1], **R. D. Badgaiyan***[2], **J. Westman**[2], and **F. Nyberg**[3]

[1] Department of Clinical Neurophysiology, University Hospital, Uppsala, Sweden
[2] Laboratory of Neuroanatomy, Department of Medical Cell Biology, Biomedical Centre, Uppsala University, Uppsala, Sweden
[3] Department of Pharmaceutical Biosciences, Division of Biological Research on Drug Dependence, Biomedical Centre, Uppsala University, Uppsala, Sweden

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Summary. The influence of exogenous rat growth hormone on spinal cord injury induced alterations in spinal cord evoked potentials (SCEP) and edema formation was examined in a rat model. Repeated topical application of rat growth hormone (20 μl of 1 μg/ml solution) applied 30 min before injury and at 0 min (at the time of injury), 10 min, 30 min, 60 min, 120 min, 180 min, and 240 min, resulted in a marked preservation of SCEP amplitude after injury. In addition, the treated traumatised cord showed significantly less edema and cell changes. These observations suggest that growth hormone has the capacity to improve spinal cord conduction and attenuate edema formation and cell injury in the cord indicating a potential therapeutic implication of this peptide in spinal cord injuries.

Keywords: Amino acids – Growth hormone – Spinal cord injury – Edema formation – Spinal cord evoked potentials – Spinal cord edema – Cell injury

Introduction

Spinal cord injury is a serious clinical condition which depending on the magnitude and severity of the primary insult can induce paralysis making life miserable and inflicting a heavy financial burden to the society (Schwab

* Present address: Harvard University, Harvard Medical School and Massachusetts General Hospital, William James Hall, Rm. 875, 33 Kirkland Street, Cambridge, MA 02138, U.S.A.

and Bartholdi, 1996; Stålberg et al., 1998). Thus efforts should be made to find out several ways to minimise the consequences of spinal cord injury in order to enhance the quality of life of the spinal cord trauma victims.

In many European, American or Australasian population, the incidence of spinal cord injury occurs at about 30 to 50 cases per million per year which mainly comprise motor vehicle accidents (Winkler et al., 1998). Other cases of spinal cord injury results from falls, gun shots, knife wounds and/or spinal cord tumours (Stålberg et al., 1998). Although, our understanding on the pathophysiology of spinal cord injury has greatly expanded in the last few years, a suitable therapeutic approach to minimise spinal cord injury induced neurological dysfunction and/or neurodegenerative changes has still not been developed. Thus, further studies in this direction are highly warranted.

Recently, it has been shown that many spinal cord trauma victims exhibit wide variety of neuroendocrine alterations involving changes in several pituitary hormone levels in the blood plasma (Bauman et al., 1994; Huang et al., 1995). One such pituitary hormone is growth hormone which is released under normal conditions regularly in the blood plasma as well as under increased physical activity (Cruse et al., 1996; Tsitouras et al., 1996). There are evidences that spinal cord injury patients lack the release of growth hormone under normal conditions and during other physical activity (Bauman et al., 1994; Cruse et al., 1996). Thus, the growth hormone levels decline in the plasma of spinal cord injured victims. The functional significance of such finding is not well understood.

It seems quite likely that release of growth hormone is somehow related with the normal cell energy metabolism and may have some beneficial effects on the CNS function. A natural decrease in release of growth hormone occurs during ageing (Bauman et al., 1994; Hanci et al., 1994). It is proposed that a decline in growth hormone level is somehow related with the neurodegenerative changes with advancing age. A decrease in growth hormone release and its low concentration in the plasma following spinal cord injury seems to be associated with the neurodegenerative changes in the spinal cord commonly seen in trauma victims. Therefore, a possibility exists that exogenous supplement of growth hormone will induce some beneficial effects in the spinal cord following trauma.

Keeping these views in consideration, the present study was undertaken to evaluate the effects of growth hormone in spinal cord trauma induced pathophysiological changes in the cord. Previously, our findings indicate that early changes in the spinal cord evoked potentials (SCEP) is a sensitive indicator of later development of spinal cord edema and cell injury and there is a strong correlation between decrease in SCEP amplitude with later development of spinal cord edema (Sharma et al., 1991). Thus, in the present investigation we evaluated the effect of rat growth hormone on spinal cord induced alterations in SCEP changes and correlated with the development of spinal cord edema at 5 h after the injury in our rat model.

Materials and methods

Animals

Experiments were carried out on 20 male Sprague Dawley rats (body weight 300–350 g) housed at controlled room temperature with 12 h light and 12 h dark schedule. Rat food pellets and tap water were provided ad libitum.

Spinal cord injury

Under Equithesin anaesthesia (3 ml/kg, i.p.), one segment laminectomy (T10-11) was made. The spinal cord injury was inflicted by making a longitudinal incision into the right dorsal horn (Sharma et al., 1991; Winkler et al., 1998a,b). The wound was covered with cotton soaked saline in order to prevent a direct exposure of the cord to air. Normal animals served as controls. This experimental condition is approved by the Ethical Committee of Uppsala University, Uppsala, Sweden and the Banaras Hindu university, Varanasi, India.

Recording of SCEP

Spinal cord evoked potentials were recorded from the epidural electrodes placed over the right dorsal surface of the spinal cord of the T9 (rostral) and the T12 (caudal) segments following stimulation of right tibial and sural nerves as described earlier (Sharma et al., 1991). The reference electrodes were placed in corresponding paravertebral muscles of the active exploratory epidural electrodes. The ground electrode was placed over the proximal end of the tail (Sharma et al., 1991; Winkler et al., 1998b).

Treatment with rat growth hormone

The rat growth hormone (rGH, Kåbi Pharmacy, Stockholm, Sweden) was applied topically over the exposed surface of the spinal cord (Sharma et al., 1998a,b). Application of rGH (20 μg/kg for 30 sec) was initiated 30 min before injury followed by 0 min (at the time of injury), 10 min, 30 min, 60 min, 120 min, 180 min and 240 min after trauma (Sharma et al., 1998b) and the SCEP was recorded using standard protocol (Sharma et al., 1991). In controls, the treatment schedule was exactly followed except the animals were not injured.

Spinal cord edema

At 5 h, spinal cord tissue containing the injured (T10-11) segments was taken out to determine edema formation by measuring water content (Sharma et al., 1998a,b; Sharma, 1999). In brief, the wet tissue was weighed immediately and placed in a oven at 90°C for 72 h in order to evaporate the tissue water. The dry weight of the sample was taken to calculate the lost water and the water content was calculated according to the formula: wet weight (mg)- dry weight (mg)/wet weight (mg) $\times$ 100 (Sharma, 1999).

Statistical analysis

ANOVA followed by Dunnet's test or unpaired Student's t-test were applied to determine the statistical significance of the data obtained. A p-value less than 0.05 was considered to be significant.

Results

Effects of rat growth hormone on SCEP changes

A focal trauma to the rat spinal cord produced by incision of the right dorsal horn at T10-11 segment resulted in an immediate depression of SCEP amplitude (mean depression 60%) which lasted for about 1 h. At the end of 5 h there was some recovery in SCEP amplitude whereas the latency of SCEP continued to increase (Fig. 1).

Application of rGH (20 μl of a 1 μg/ml solution for 30 sec) resulted in a marked protection of SCEP amplitude seen after injury (Fig. 2). Thus in the growth hormone treated rats, the SCEP amplitude did not diminish after trauma (Fig. 2).

Effects of rat growth hormone on spinal cord edema

A focal trauma to the rat spinal cord significantly increased the spinal cord water content by more than 3% from the control value (Fig. 3). Treatment with rat growth hormone markedly attenuated this increase in spinal cord water content in the traumatised rats at 5 h (Fig. 3). However, treatment with growth hormone alone in normal rats did not influence the spinal cord water content.

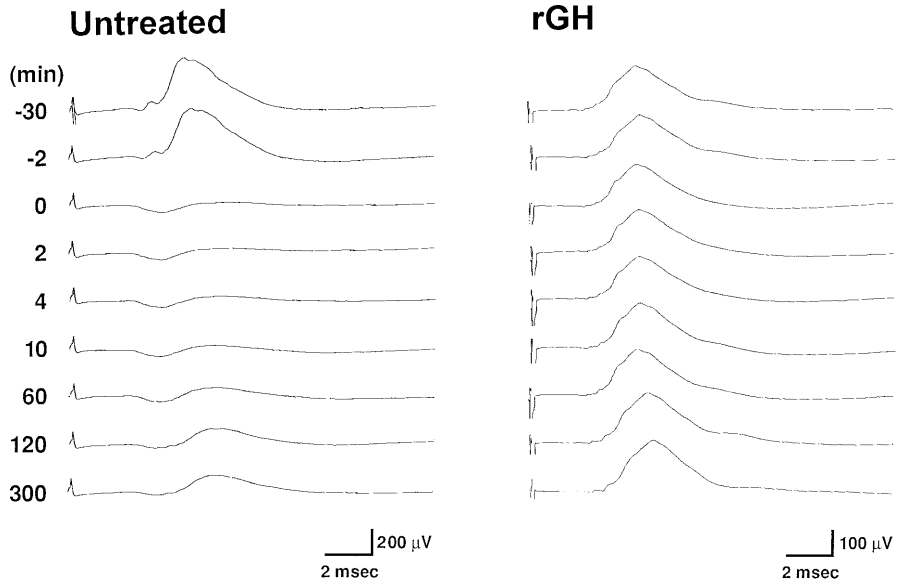

Fig. 1. A representative example of SCEP recordings from one untreated (left) and one rat growth hormone (*rGH*) treated (right) spinal cord traumatised animals. Spinal cord injury was produced at 0 min and the rGH was applied in treated rats 30 min before injury (−30 min)

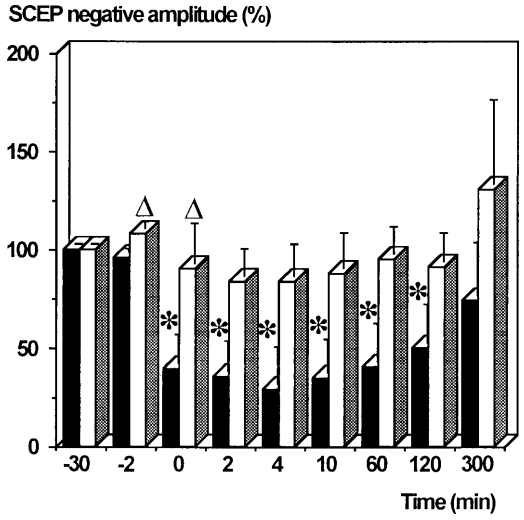

Fig. 2. Mean negative SCEP amplitude in untreated (black bars) and rat growth hormone treated (blank bars) animals. * = $P < 0.05$ from −30 min; Δ = $P < 0.05$ from untreated group; ANOVA followed by Dunnet's test for multiple group comparison

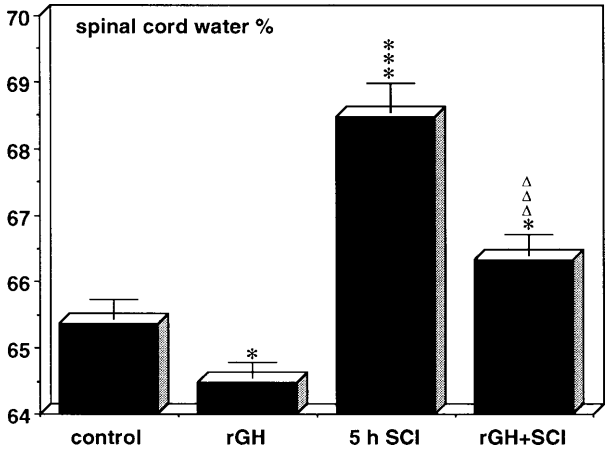

Fig. 3. Spinal cord water content in control or spinal cord injured rats and its modification with rat growth hormone (*rGH*) pretreatment. * = $P < 0.05$, *** = $P < 0.001$, compared from control group; ΔΔΔ = $P < 0.001$ compared from spinal cord injured (*SCI*) group, Student's unpaired t-test

Discussion

Spinal cord evoked potentials (SCEP) is altered immediately following lesion of the spinal cord. The changes in the SCEP mainly represent a decrease in negative amplitude and an increase in latency of the negative peak (Sharma et al., 1991). In case of severe injury, appearance of positive peak on the rostral recording is a quite common phenomenon which represents injury potential (Sharma et al., 1991; Winkler et al., 1998a,b). There are reports that loss of negative peak and appearance of positive peak is related to the local

neurochemical metabolism immediately following trauma (Sharma et al., 1991; Winkler et al., 1998a). Thus, pretreatment with several drugs which are able to modify the neurochemicals involved in the secondary injury cascade will attenuate SCEP changes and improve spinal cord conduction. Accordingly, inhibition of serotonin synthesis or prostaglandin synthesis significantly improve the spinal cord conduction following trauma and thwart the later development of spinal cord pathophysiology (Sharma et al., 1991, 1997, 1998b; Stålberg et al., 1998; Winkler et al., 1998a,b).

The present results for the first time suggest that exogenous application of growth hormone on the spinal cord following injury is neuroprotective in nature. Our results further show that growth hormone application has the capacity to improve spinal cord conduction following injury suggesting that the peptide is able to modify the secondary injury cascade, not reported earlier. It appears that either growth hormone is modulating the neurochemical cascade following spinal cord injury or may have a direct beneficial effects on the pathophysiology of cord trauma (Schwab and Bartholdi, 1996). The detailed contribution of growth hormone in the pathophysiology of spinal cord injury is not known.

Growth hormone is necessary for cell metabolism and a regular release of the peptide is essential for normal activity of the CNS (Bauman et al., 1994; Cruse et al., 1996; Winkler et al., 1998a). The growth hormone is known to bind IGF-1 receptors in the CNS (Tsitouras et al., 1995; Sharma et al., 1997). The IGF-1 belongs to the neurotrophins family and possesses neuroprotective effects if supplied exogenously following several types of CNS insults (Rodriguez et al., 1998; Tonra, 1999; van Ooyen and Wilshaw, 1999). A possibility exists that stimulation of IGF-1 receptors is somehow associated with neuroprotection (Sharma et al., 1997, 1998a,b). This idea is well supported by the findings of a downregulation of IGF-1 and IGF-2 receptors in several neurodegenerative diseases such as in Alzheimer's disease, chronic neurological dysfunction and dementia (Schwab and Bartholdi, 1996).

Recent evidences suggest that growth hormone level is significantly declined in patients following spinal cord injury (Bauman et al., 1994; Hanci et al., 1994; Huang et al., 1995). However, it is not yet certain that the number of growth hormone receptors or its binding sites are also downregulated in the CNS following trauma. This is a new subject which requires further investigation. A decrease in growth hormone means that cellular energy metabolism is impaired in the CNS which may itself trigger various neurodegenerative changes in the cord. Exogenous supplement of the growth hormone may enhance the cellular activity following trauma and maintain the cellular energy levels adequately so that the cell injury is either delayed or abolished in growth hormone treated and traumatised rats. Since our study is limited to only 5 h survival period, it seems that application of rat growth hormone in spinal trauma has perhaps delayed the onset of cellular changes. To further clarify this, additional studies using prolonged survival periods of 12 h to 24 h following trauma are needed. These investigation would clarify whether the

beneficial effects of growth hormone treatment only last for a shorter duration or whether these changes are sustainable for longer periods making the peptide suitable for future therapeutic strategy in spinal cord injury.

We have applied a relatively high concentration of the growth hormone over the spinal cord. In general, vascular permeability of the rat growth hormone across the CNS is quite limited (Mustafa et al., 1995). Thus under normal conditions growth hormone does not permeate in the CNS compartment if administered intravenously. However, a significant increase in growth hormone permeability occurs following spinal cord injury (Mustafa et al., 1995). In our laboratory we have seen a marked increase in the growth hormone permeability following 5 h spinal cord injury in regions far beyond the lesion site (Winkler et al., 1998a). This indicates that application of growth hormone over the traumatised cord will enhance the delivery of the peptide in the CNS. An increased permeability of the BSCB will allow growth hormone to penetrate into the spinal cord deeper and to influence cellular energy metabolism.

Improved spinal cord function and conduction following injury mainly depends on the spinal cord injury induced "shock phase" response which seems to be mediated via several neurochemicals (Sharma et al., 1991; Winkler et al., 1998a). There is ample evidence in the literature that following trauma to the cord, many nerve cells stop conducting because they enter in the "shock phase" which lasts for 60 to 90 minutes (Stålberg et al., 1998). It may be that growth hormone attenuates the cellular shock phase. Thereby an improved spinal cord conduction is maintained after injury (Westman and Sharma, 1998; Sharma et al., 1998a,b). To further clarify this field mapping studies are needed.

Our study further demonstrates that growth hormone has attenuated spinal cord edema formation seen 5 h after injury. Since growth hormone can attenuate stress reaction of the cells, it seems likely that the magnitude and severity of the secondary injury cascade is also attenuated (Sharma, 1999). This observation is in line with our previous observations which suggest that drugs which modify or protect early SCEP changes following spinal cord injury are able to reduce the later development of the spinal cord pathophysiology and edema formation (Winkler et al., 1998a,b). Thus the present results suggest that growth hormone has the capacity to induce neuroprotection in the spinal cord following injury as reflected in the early SCEP changes and later cord pathology.

In conclusion, our results strongly point out a potential therapeutic value of growth hormone for the treatment of spinal cord injuries. However, to explore further the possibility of growth hormone as a treatment of spinal cord injury, application of the hormone at various time intervals after injury is needed. This is currently being investigated in our laboratory.

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Authors' address: Hari Shanker Sharma, Dr. Med. Sci., Laboratory of Neuroanatomy, Department of Medical Cell Biology, Biomedical Centre, Box 571, Uppsala University, SE-751 23 Uppsala, Sweden,
Fax: +46-18-24 38 99, E-mail: Sharma@medcellbiol.uu.se

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