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Repeated topical application of growth hormone attenuates blood-spinal cord barrier permeability and edema formation following spinal cord injury

An experimental study in the rat using Evans blue, [125]I-sodium and lanthanum tracers

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Summary. The neuroprotective efficacy of growth hormone on a focal spinal cord trauma induced alteration in the blood-spinal cord barrier (BSCB) and edema formation was examined in a rat model. Under Equithesin anaesthesia, one segment laminectomy was done over the T10–11 segments. Spinal cord injury was produced by making an incision into the right dorsal horn of the T10–11 segments (2 mm deep and 4 mm long). The animals were allowed to survive 5 h after injury. Highly purified rat growth hormone [rGH, 25 μl of a 1 μg/ml solution) was applied over 10 sec topically on the exposed surface of the spinal cord 30 min before injury. The identical doses of the rGH were repeated 0 min, 30 min, 60 min, 120 min, 180 min and 240 min following injury. Saline (0.9% NaCl) treated traumatised rats at identical intervals served as controls. Traumatised rats treated with saline exhibited marked edema formation and extravasation of Evans blue and [125]Iodine tracers in the spinal cord. At the ultrastructural level, perivascular edema and exudation of lanthanum across the endothelial cells was quite frequent in the spinal cord. Pretreatment with rGH significantly attenuated the edema formation and the extravasation of tracers in the spinal cord. In these rats, perivascular edema and infiltration of lanthanum across the endothelial cells was not much evident. These observations show that the rGH has the capacity to reduce the early manifestations of microvascular permeability disturbances and edema formation following trauma and further suggest a possible therapeutic potential of the hormone for the treatment of spinal cord injuries.

Keywords: Blood-spinal cord barrier (BSCB) – Edema – Laminectomy – Microvascular permeability – Rat – Rat growth hormone (rGH) – Spinal cord injury – Topical application – Visual swelling

Introduction

The blood-spinal cord barrier (BSCB) strictly regulates the fluid microenvironment of the spinal cord within a narrow limit (Schwab and Bartholdi 1996; Sharma et al., 1990, 1993, 1995a,b). Alterations in the BSCB seen in many experimental and clinical situations are often associated with abnormal function of the spinal cord (Juhler 1984; Popovich et al., 1996; Stålberg et al., 1998; Tator and Fehlings 1991). There are reasons to believe that the microvascular permeability disturbances play prominent roles in vasogenic edema formation and cell injury following several kinds of noxious insults to the brain or spinal cord (Sharma et al., 1998a–c; Winkler et al., 1998; Sharma 1999, 2000).

Previous studies have shown that a focal trauma to the spinal cord induces widespread disturbances in the microenvironment of the spinal cord far from the site of the primary insult (Olsson et al., 1990; Sharma et al., 1993, 1995a, 1998c). Interestingly, pretreatment with several drugs known to modify the neurotransmission of e.g. serotonin, prostaglandin and the endogenous opioids are able to attenuate the BSCB permeability disturbances (Olsson and Sharma 1990; Sharma et al., 1995a,b; Olsson et al., 1995). These observations indicate that the microvascular permeability disturbances are mediated by several neurotransmitters or neuromodulators that can be influenced by suitable pharmacological agents.

Growth hormone (GH) is recognised as an important polypeptide hormone known to influence immune, endocrine and metabolic functions of the spinal cord (Cruse et al., 1996; Bauman et al., 1994). The amount of GH is drastically reduced in victims of spinal cord injury (Bauman et al., 1994; Tsitouras et al., 1995; Huang et al., 1995). This indicates that the hormone participates in some of the consequences of spinal cord injury, the details of which are not yet well known.

The GH is produced in the anterior pituitary and released into the circulation to reach its receptors located on the cell surface on target organs present in the periphery (Nyberg 1997; Kopchick and Andry, 2000). However, recent studies have suggested that GH may penetrate the blood-brain barrier (BBB) to reach areas in the brain and spinal cord responsive to the hormone (for review, see Nyberg, 2000). Following GH replacement therapy, the hormone concentration was found to increase in the extracellular fluid of the central nervous system (CNS) as well as in the cerebrospinal fluid (CSF) (Johansson et al., 1995). Furthermore, the presence of specific receptors for GH in certain areas of the brain (Nyberg and Burman, 1996; Nyberg, 2000) and spinal cord (Thörnwall-Le Grevés et al., 2001) has recently been demonstrated.

The recent works of Scheepens et al. (2001) show that GH may serve as a rescue factor during recovery from CNS injury in the rat. Thus, an increased GH-like immunoreactivity in the brain occurs few days after the hypoxic-ischemic injury. Furthermore, treatment with GH soon after the injury reduces the extent of neuronal loss. The authors suggest that a neural GH axis is activated during recovery of injury and that this may act to restrict the extent of neuronal damage.

There is one previous report by Hanci and coworkers (1994) indicating that prolonged treatment with biosynthetic GH attenuates some of the neurological motor dysfunction seen 3 weeks after trauma following clip compression induced spinal cord injury. These authors attributed this positive effect of GH to enhanced regeneration capabilities of the peptide within the spinal cord. Moreover, in a study using transgenic mice it was shown that GH over expression influences the spinal motoneurons size in conjunction with whole body size. The hormone was found to increase nucleolar, nuclear, and cell body size in lumbar spinal motoneurons (Chen et al., 1997). This observation suggests that GH may have a regulatory function on spinal motoneurons.

In victims of spinal cord injury, the daily secretion of GH and insulin like growth factor-1 (IGF-1) are known to be decreased in young subjects, less than 45 years old (Bauman et al., 1994). Low levels of IGF-1 and GH in these trauma victims are adversely influencing their ambulatory conditions and can lead to premature ageing. Thus, GH and IGF-1 replacement therapy seem to be a promising approach in the future treatment of patients with spinal cord injury.

Keeping these views in consideration, our laboratory has shown that topical application of IGF-1 on the traumatised spinal cord significantly attenuated edema formation and cell damage in the spinal cord (Sharma et al., 1997a,b; 1998b,c). However, the outcome of exogenous supplement of GH in spinal cord injury is in general not yet extensively investigated. The influence of GH on the early changes in the fluid microenvironment of the spinal cord following trauma is still unknown.

The GH permeability to the spinal cord under normal conditions is quite low (Mustafa et al., 1995). An enhanced permeation of the hormone into the spinal cord can be achieved after a focal trauma to the cord (Mustafa et al., 1995). Thus, it appears that topical application of the polypeptide may be a suitable approach to study the effects of GH on the early trauma-induced pathophysiological consequences.

The present investigation was carried out to examine whether repeated topical application of GH can influence trauma-induced alterations in the microvascular permeability disturbances and edema formation in the cord. In addition, whether the influence of the hormone can be seen in far remote regions of the cord not directly involved by the primary physical lesion.

Materials and methods

Animals

Experiments were carried out on 95 male Sprague Dawley rats (Alab, Stockholm) weighing between 220–280 g (age 18 to 20 weeks). The animals were housed at controlled room temperature (21 ± 1°C) with 12 h light and 12 h dark schedule. Food and water before the experiments were supplied *ad libitum*.

Spinal cord injury

Spinal cord injury was performed under Equithesin anaesthesia (3 ml/kg, i.p.). After incision of the back skin, one segment laminectomy was done at the T10–11 segment. Infliction of trauma was made by making an incision into the right dorsal horn (about 2 mm deep and 4 mm long) using a sterile scalpel blade (Sharma and Olsson, 1990). The wound was subsequently covered with cotton soaked in saline. These animals were allowed to survive 5 h after

injury and the anaesthesia was properly maintained by repeated injection of equithesin at lower dosages (1 ml/kg, i.p.) at every 1 h interval. The equithesin anaesthetised, intact animals were used as controls. These experimental conditions were approved by the Ethics Committee in Uppsala.

Treatment with rat growth hormone (rGH)

The highly purified rGH (Roos et al., 1987) was dissolved in saline to yield a concentration of 1 μg/ml. Using a microliter syringe (Hamilton, UK) 25 μl of this solution was applied in 10 sec over the exposed surface of the spinal cord (Sharma et al., 1995a, 1997a,b). Thirty min after application of rGH the spinal cord injury was made. Identical dose of the hormone was applied repeatedly, 2 min after injury followed by 30 min, 60 min, 120 min, 180 min and 240 min after trauma. The animals were sacrificed at the end of 5 h after injury.

In a separate group of rats rGH was applied topically on the exposed spinal cord after laminectomy in the identical manner as described above. However, in these animals no spinal cord injury was made. These animals were used as rGH treated controls (Table 1).

Treatment with physiological saline

In separate groups of rats, physiological saline (0.9% NaCl) was applied in animals either subjected to laminectomy alone or 5 h spinal cord injury. The treatment schedule with saline was identical to rGH as mentioned above (Table 1).

Table 1. Schedule for treatment with rat growth hormone (rGH) in control and experimental animals

		Experiment type laminectomy		Spinal cord injury	
	controls	saline	rGH	saline	rGH
BSCB	5	5	6	5	6
Water content	5	5	6	6	6
Lanthanum	6	–	–	6	6
Physiological variables	4	4	5	4	5
Total	20	14	17	21	23

Microvascular permeability

Microvascular permeability was examined using $^{[125]}$Iodine as a protein tracer according to the standard protocol (Sharma 1987; Sharma et al., 1993 and 1995a). In brief, radioactive material (about 10^6 counts per minute) dissolved in 0.5 ml saline was administered intravenously through arterial puncture in the right femoral vein 5 min before termination of the experiment. The radiotracer was allowed to circulate for 5 to 8 min in the blood stream. The intra-arterial tracer was washed out by a brief saline rinse through heart (0.9% saline for 45 sec). Immediately before the saline wash one ml of whole blood was taken out after cardiac puncture for estimation of the whole blood radioactivity (Sharma et al., 1990).

To understand the influence of local circulatory disturbances following trauma due to ischemia and its influence on radiotracer trapping, serial samples of arterial blood were withdrawn. Blood was collected at a rate of 0.8 ml/min from the femoral artery in 3 animals of each group starting from 30 min before radiotracer injection followed by 30 sec, 60 sec, 2 min, 4 min and 5 min after injection of the radiotracer (Sharma and Dey, 1987). The result of this investigation is shown in Fig. 1. It appears from the figure that distribution of radiotracer in the circulation was homogenous irrespective of the trauma or control group.

Immediately after saline perfusion, the spinal cord and brain was dissected out and placed in ice cold saline in a Petri dish. Large superficial vessels over the brain or spinal cord, if any, were discarded. Small pieces of the spinal cord tissue (C5, T4, T9, T10–11 and T12) and brains (cerebral cortex, hippocampus, cerebellum, hypothalamus and brain stem) were dissected out (sample size ranging from 85 mg to 150 mg), placed in tarred vials and counted in a 3-in well type gamma counter at 50–80 KeV (Sharma and Dey, 1987). Extravasation of radioactivity in tissue is expressed as percentage increase over blood radioactivity using the formula CPM/g. tissue/ CPM/g.blood $\times$ 100 as described earlier (Sharma et al., 1990).

Lanthanum extravasation at the ultrastructural level

In separate group of 10 spinal cord traumatised rats treated with either saline (n = 5) or rGH (n = 5), extravasation of lanthanum (an

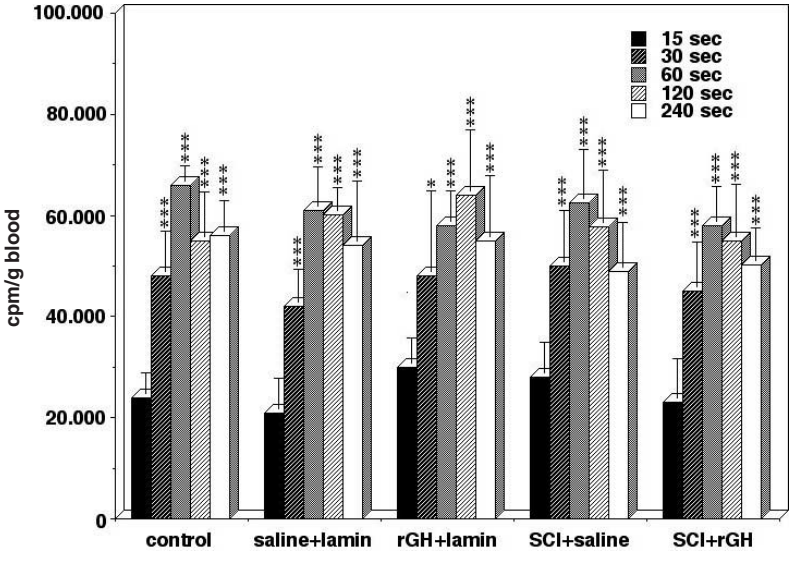

Fig. 1. Timed arterial concentration of the radiotracer $^{[125]}$Iodine (expressed in cpm) injected into the right femoral vein in control, laminectomised, spinal cord injured rats and its modification with saline or growth hormone treatment. Note that no significant difference in any group exists in tracer distribution. Tracer concentration in the plasma increased significantly from the 15 sec and onwards sample and reached its plateau at 60 sec irrespective of the trauma or treatment group. *** $P < 0.001$, Student's paired t-test

Table 2. Physiological variables in control, saline and rat growth hormone treated 5 h lameniectomised or spinal cord injured rats

Type of experiment	n	MABP torr	Arterial pH	PaO_2 torr	$PaCO_2$ torr
Control	4	108 ± 6	7.38 ± 0.04	81.58 ± 0.32	34.48 ± 0.12
Laminectomy + saline	4	98 ± 5	7.33 ± 0.02	79.12 ± 0.56	33.32 ± 0.21
Spinal cord injury + saline	5	88 ± 4**	7.30 ± 0.06	78.34 ± 0.44	32.39 ± 0.48
Laminectomy + rGH	4	94 ± 6**	7.32 ± 0.04	78.56 ± 0.32	33.08 ± 0.59
Spinal cord injury + rGH	5	92 ± 5**	7.33 ± 0.06	78.89 ± 0.61	32.92 ± 0.54

Values are Mean ± SD; ** = $P < 0.01$, Student's unpaired t-test, compared from control

Table 3. Spinal cord water content and volume swelling in control, saline and rat growth hormone treated 5 h lameniectomised or spinal cord injured (SCI) rats

Type of experiment	n	T9 Water content %	*f*#	T12 Water content %	*f*#
Control	5	65.34 ± 0.33	–	65.63 ± 0.23	–
Laminectomy + saline	5	66.23 ± 0.21*	+2	66.48 ± 0.18*	+2
Laminectomy + rGH	5	65.78 ± 0.12	–	65.81 ± 0.16	–
Spinal cord injury + saline	6	68.67 ± 0.34***	+16	68.89 ± 0.23***	+16
Spinal cord injury + rGH	5	66.76 ± 0.23**[a]	+6	66.86±0.28**	+6

Values are Mean ± SD; *f*# = volume swelling calculated according to Elliott and Jasper (1949) * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ compared to control; [a] = $P < 0.01$ compared to SCI

electron dense tracer; Olsoon et al., 1990; Sharma et al., 1993) was examined at the ultrastructural level as described earlier (Sharma et al., 1991). In brief, the lanthanum chloride (2.5%) was dissolved in 0.4% paraformaldehyde in 0. 1 M phosphate buffer saline (pH 7.0). At the end of 5 h after spinal cord injury, the animals were perfused with the fixative containing lanthanum via heart preceded with a brief saline rinse (Sharma et al., 1991). After the perfusion, the spinal cord segments from the T9, T10–11 and the T12 segments were dissected out. The small tissue pieces from these segments were post-fixed in osmium tetraoxide, embedded in Epon 812 and processed for standard transmission electron microscopy (Sharma et al., 1990, 1991, 1993). The ultrathin sections were contrasted with uranyl acetate and lead citrate (Sharma et al., 1991). The lanthanum deposits in these ultrathin sections can be seen as dark electron dense particles either confined within the lumen of the microvessels or infiltrated into the endothelial cell cytoplasm and in basal lamina (Sharma et al., 1993).

Edema formation and volume swelling

Edematous swelling of the spinal cord was examined using naked eye inspection with the help of a magnifying lens in control, laminectomised or spinal cord injured rats treated with either saline or rGH (Sharma et al., 1995a,b and 1996). After examination of the visual swelling the spinal cord samples comprising the T9 and the T12 segments were weighed immediately and placed in an oven maintained at 90°C for 72 h to determine their dry weights. The difference between the wet weight and the dry weight of the cord tissue was used to calculate water content of the sample (Sharma and Olsson, 1990). The volume swelling of the spinal cord segments was determined according to the Elliott and Jasper (1949). In general, an increase in 1% of the spinal cord water content will represent a 4% volume swelling of the cord (Sharma and Olsson, 1990) (Table 3).

Physiological variables

In a separate group of rats (Table 1) arterial blood gases and mean arterial blood pressure (MABP) was monitored in controls, saline or GH treated laminectomised or spinal cord injured rats (Table 2). In brief, MABP was monitored from a cannula (PE 10) placed into the right carotid artery (Sharma et al., 1990). At the time of recording of MABP the arterial cannula was connected to a pressure transducer (Statham, Strain gauge, USA) and the output was fed to a chart recorder (Electromed, UK). Immediately before monitoring of the MABP, samples of arterial blood were withdrawn to monitor blood gases in a Radiometer apparatus (Copenhagen).

Statistical analysis

Student's unpaired t-test was used to evaluate the statistical significance of the data obtained. A p-value less that 0.05 was considered significant.

Results

Effect of rGH on physiological variables

Laminectomy alone did not influence the MABP, arterial pH and blood gases significantly compared to the control group. However, subjection of rats to a focal trauma significantly attenuated the MABP by about 20 torr. This effect of trauma on the MABP was not influenced by treatment with the rGH (Table 2). The other physiological variables such arterial pH and

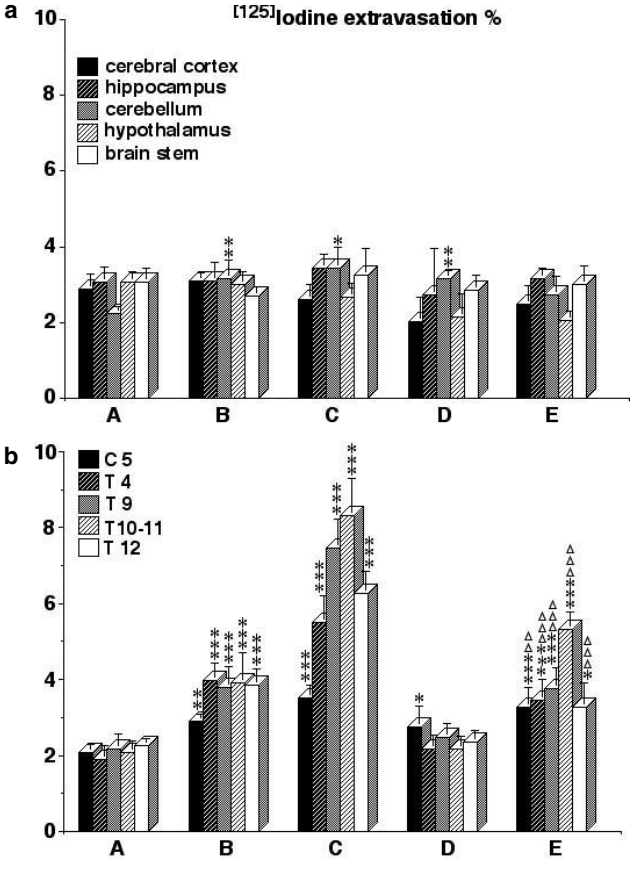

Fig. 2. Extravasation of [125]Iodine in 5 brain regions (**a**) and in 5 different spinal cord segments (**b**) in control, laminectomised or spinal cord injured rats and their modification with saline or growth hormone treatment. * = $P < 0.05$, ** = $P < 0.01$, *** = 0.001, Student's unpaired t-test compared from control group. A = control; B = saline + laminectomy; C = SCI; D = rGH + laminectomy; E = SCI + rGH

blood gases were not significantly affected in either saline or rGH treated groups following trauma (Table 2).

Effect of rGH on microvascular permeability

A faint blue staining of the exposed spinal cord was observed in the 5 h laminectomised group subjected to saline treatment. This staining in Evans blue was considerably reduced by rGH treatment. Measurement of the radiotracer in the laminectomised group exhibited a mild but significant increase in the [125]Iodine extravasation in most of the spinal cord segments at 5 h (Fig. 2). Treatment with rGH in these laminectomised animals significantly attenuated the radiotracer extravasation in the cord (Fig. 2a).

A focal trauma to the spinal cord profoundly increased the BSCB permeability to Evans blue and the [125]Iodine into the spinal cord. The blue staining was most prominent in the traumatised segment. The adjacent T9 and the T12 segments exhibited a mild to moderate blue staining. No visible Evans blue staining was seen in the C5 segment and T4 segments after injury. On the other hand, about 6–7 fold increase in the radiotracer was observed in the spinal cord segments close to the injury site (Fig. 2). However, the far remote regions of the spinal cord segment C5 and T4 also showed a mild but significant increase in the radioactivity (Fig. 2).

Pretreatment with rGH significantly attenuated the Evans blue and radiotracer extravasation in the spinal cord following trauma. Thus the intensity of Evans blue staining is considerably reduced in the T10–11 segments. This effect was most pronounced in the adjacent T9 and the T12 segment of the traumatised spinal cord. The rGH also significantly reduced the extravasation of the radiotracer in all the spinal cord segments examined. However, the most marked effect of the rGH on the reduction in tracer extravasation was seen in the segments located far from the injured site (Fig. 2). Thus, the extravasation of the radiotracer was only minimal in the C5, T4, T9 and T12 segments (Fig. 2a).

On the other hand, in this study we did not observe any significant increase in iodine extravasation in the brain except a mild increase in the cerebellum (Fig. 2) following laminectomy or spinal cord injury (Fig. 2). Interestingly, treatment with rGH significantly attenuated the spinal cord injury induced increase in the radiotracer extravasation in cerebellum (Fig. 2). In other regions of the brain the distribution of the radiotracer was almost similar in all groups of the rats examined (Fig. 2).

Effect of GH on edema formation

A focal trauma to the spinal cord resulted in marked visual swelling of the cord, which often bulged out from the laminectomised area indicating a profound edema formation and volume swelling of the cord. Measurement of the water content revealed a 4% increase in the water content corresponding to a 16% increase in volume swelling in the saline treated rats. Pretreatment with rGH significantly attenuated the increase in water content and the volume swelling (Table 3).

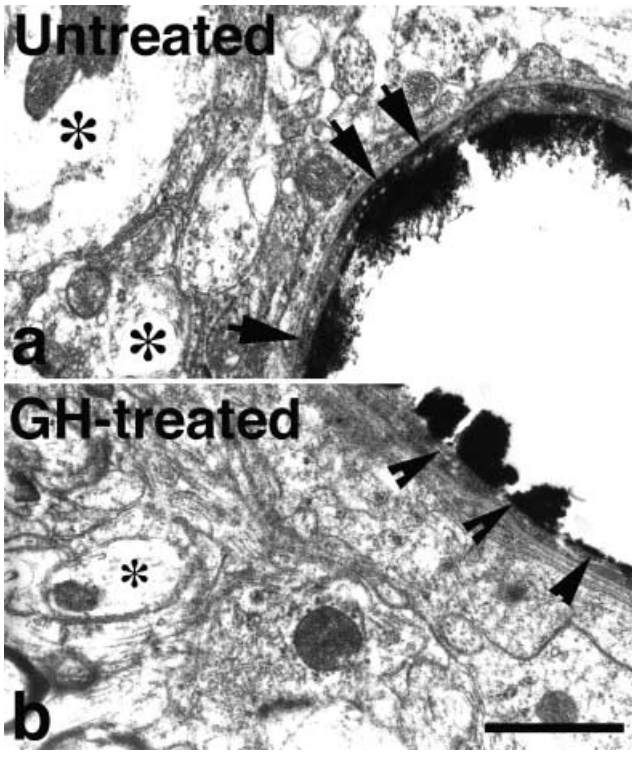

Fig. 3. High power transmission electron-micrograph showing lanthanum (dark black particles) extravasation in the dorsal horn of the T9 segment of one saline treated traumatised rat (**a**) and its modification with the rGH treatmenst (**b**). The exudation of lanthanum in the rGH treated rat is extensively reduced. Signs of perivascular edema and myelin vesiculation (*) are considerably reduced in the rGH treated animal (bar = 200 nm)

A mild increase in water content and volume swelling can also be seen in the laminectomised group. This increase in the water content and volume swelling was significantly reduced by the rGH treatment (Table 3).

Ultratstructural studies of lanthanum extravasation

Ultrastructural studies using lanthanum showed marked infiltration of the tracer into the endothelial cell cytoplasm of the traumatised rats treated with saline. The lanthanum tracer was also seen in the basal lamina of many microvessels. Signs of perivascular edema, membrane disruption and myelin vesiculation are prominent in this group (Fig. 3).

On the other hand, pretreatment with rGH significantly attenuated the infiltration of lanthanum into the endothelial cell cytoplasm as well as into the basal lamina (Fig. 3). In these rGH treated and traumatised animals, the lanthanum tracer is mainly confined within the lumen. In this group, perivascular edema, myelin vesiculation and membrane disruption was much less frequent (Fig. 3).

Discussion

The result emerged from the present study show that rGH when applied repeatedly in high quantities has the capability to alleviate early disturbances in the fluid microenvironment of the spinal cord and edema formation after a traumatic insult. This is evident from the observation that the extravasation of Evans blue and the radiolabelled iodine tracers are significantly reduced in the spinal cord of traumatised rats treated with the hormone. The rGH was also able to significantly thwart the edema formation in the traumatised cord. Our data thus suggest a potential therapeutic role of the GH in the early manifestations of spinal cord injuries, not reported earlier.

Previously, Hanci et al. (1994) examined the effect of long-term treatment with GH on spinal cord clip compression-induced motor dysfunction. They found a slight improvement in motor function 3 weeks after the hormone treatment. Clip compression is a severe form of injury, which often induces complete paralysis (Tator and Fehlings, 1991). In these models, drugs, when given after compression, usually do not show any significant improvement of the neurological functions (Schwab and Bartholdi, 1996). However, the findings of Hanci et al. (1994) strongly support our observations that GH has some beneficial effects on the consequences of spinal cord injury.

We have chosen a short survival period to study the effect of GH on the consequences of traumatic spinal cord injury. The early consequences of trauma seem to play a significant role in predicting the later cord pathology (Sharma et al., 1991; Stålberg et al., 1998). This is supported by the fact that a drug given within 8 h period of traumatic insult is able to produce some beneficial effects on the neurological outcome (Faden, 1993). However, if the same drug is applied after longer intervals, no beneficial effect can be seen (Schwab and Bartholdi, 1996; Sharma et al., 1998b,c; Winkler et al., 1998). Accordingly, it seems highly warranted to study the early consequences of spinal trauma and to limit these events with pharmacological manipulation (cf Winkler et al., 1998). An early reduction in trauma induced BSCB and edema formation by rGH is quite promising to explore the future therapeutic possibilities of the hormone in clinical settings.

A reduction in the magnitude and intensity of the BSCB breakdown and edema formation by the rGH treatment indicate that the hormone has the capacity to influence the early releases of chemicals and/or secondary injury factors following trauma. GH deficiency occurs in patients suffering from spinal cord injuries (Bauman et al., 1994; Tsitoras et al., 1995). The functional significance of such finding is not detaily known. It seems likely that the influence of GH on local cellular metabolism, endocrine functions and immunological reactions within the cord may play important roles (Cruse et al., 1996). Obviously, an exogenous supplement of the polypeptide may have a beneficial effect.

Local application of GH can yield high concentration of the hormone within the spinal cord. Drugs, chemicals and antibodies can gain access within the cord in a relatively short period of time, if applied topically on the spinal cord (Sharma et al., 1995b, 1997b, 2000a,b). This is evident with our previous findings that topical applications of dynorphin, serotonin and nitric oxide synthase antibodies are able to attenuate trauma-induced BSCB permeability disturbances, edema formation and cell changes (Sharma et al., 1995b, 1996 and 1997b). In these studies, it was found that local application of antibodies neutralises *in vivo* tissue antigens. Thus, treatment with NOS and 5-HT antibodies significantly reduced the upregulation of NOS and 5-HT expression within the cord following trauma (Sharma et al., 1996, 1997b, 2000a,b). These observations clearly demonstrate that local application of antibodies, pharmacological agents or hormones may result in a better and direct access to the spinal cord cellular microenvironment.

It seems quite likely that opening of the BSCB permeability by focal trauma may have facilitated the transport of several chemicals, drugs and hormones within the cord (Sharma et al., 1998a). On the other hand, local swelling and poor perfusion of the traumatised cord associated with ischemia will limit the transport of chemical compounds within the cord. However, it appears from this study that chemicals can also gain access if applied over the spinal cord for some time even when the cord is not damaged. Thus, GH exhibited a significant beneficial effect in animals in which only laminectomy was performed. This indicates that spinal cord is a very sensitive organ and can be influences directly by topical application of active chemical compounds.

In this study we have focused our attention on opening of the BSCB following trauma as a key phenomenon to study the spinal cord pathophysiology. BSCB permeability is a very sensitive indicator of spinal cord function (Faden and Salzman, 1992; Schwab and Bartholdi, 1996). There are evidences that the BSCB permeability can not only be increased by focal trauma, ischemia or hypoxia but marked disturbances in this barrier can also be achieved by drugs injected in hind paw which can produce local inflammation (Gillardon et al., 1997). This is apparent from our study, which further adds that laminectomy alone is sufficient to disturb the spinal cord microenvironment resulting in a small but significant increase in tracer extravasation within the cord. Taken together, it seems quite likely that even at slight disturbances in the spinal cord peripheral or central microenvironment, the permeability of the BSCB is compromised.

A breakdown of the BSCB is directly related with extravasation of serum proteins and formation of vasogenic edema (Sharma et al., 1998a). This is clearly evident from the findings that spinal cord visual swelling and increased water content of the cord are quite distinct in saline treated animals which showed profound increase in the BSCB permeability to radiotracers and the lanthanum. On the other hand, in GH treated rats, both the edema formation and extravasation of the radiotracer were considerably reduced. This effect was also clearly seen at the ultrastructural level in which lanthanum extravasation and perivascular edema was both markedly attenuated in the rGH treated and traumatised rats. These observations are in line with the idea that a breakdown of the BSCB is primarily responsible for the vasogenic edema formation leading to the cell injury.

We did not observe extravasation of the radiotracer in any brain regions except cerebellum in the present study after spinal trauma. Previously, we have seen small but significant increase in permeability of radiolabelled rGH in some brain regions after similar trauma (Mustafa et al., 1995). This difference in tracer distribution following trauma could be related to the different types of the tracers used. Radiolabelled iodine administered into the blood stream binds to the serum proteins and thus the extravasation of iodine represents tracer-protein complex (Rapoport, 1976; Sharma et al., 1998a; Sharma 1999, 2000). Obviously, the molecular size of GH (MW = 22000) is relatively small compared to such tracer-protein complex (MW = 60000). This size difference may explain why GH

could reach the brain following similar trauma, as shown in our previous study (Mustafa et al., 1995). The tracer size appears to be the main limiting factor for Evans blue (MW 78000) extravasation in far remote regions following trauma in this study as seen in our previous investigations (Sharma and Dey, 1987; Sharma et al., 1993, 1995a, 1998a, 2000a,b).

The mechanism by which GH exerts the effects observed in this study is not clear. IGF-1 has previously been shown to elicit similar effects. Although the presence of growth hormone receptors in the brain and spinal cord, as well, is well established (Nyberg and Burman, 1996; Nyberg, 2000; Thornwall-Le Grevés et al., 2001) it is not clear whether stimulation of these sites leads to the release of IGF-1. However, as both GH and IGF-1 produce similar effects on the outcome of spinal cord trauma it is likely that the effect seen by GH is mediated by a mechanism involving a secondary release of its mediator IGF-1. However, further studies are needed to clarify the effects of GH on trauma induced ischemia and expression of key endothelial transporters and enzymes. These studies will provide a greater understanding on the cellular and molecular mechanisms of GH-induced neuroprotection, a feature currently being investigated.

Conclusion

In conclusion, our results for the first time show that rGH is capable of attenuating early manifestations of trauma induced microvascular permeability disturbances and edema formation. However, application of rGH at different time intervals after trauma is needed to explore the potential therapeutic value of the hormone in the clinical settings of the spinal cord injuries.

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