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Growth Factor Receptors in Amyotrophic Lateral Sclerosis

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Abstract

The regional distribution of nerve growth factor (NGF) and insulin-like growth factor-1 (IGF-1) receptors in human spinal cords from controls and amyotrophic lateral sclerosis (ALS) patients was studied by quantitative autoradiography. High-affinity nerve growth factor receptors were found to be distributed to a similar extent within the various segments of the human spinal cord and predominantly within the substantia gelatinosa of the dorsal horn, whereas no significant binding could be detected in the motor-neuron areas. A similar pattern of binding was obtained in the ALS spinal cords. Moreover, no reexpression of NGF receptors could be demonstrated in the motor-neuron areas of ALS spinal cords. When comparing ^{125}I-IGF-1 binding in the different spinal levels of normal spinal cord, the same distribution pattern was found in which the binding was highest in the central canal > dorsal horn > ventral horn > white matter. In the ALS cases, although a general upregulation of IGF-1 receptors was observed throughout the spinal cord, significant increases were observed in the cervical and sacral segments compared to controls. The cartography of IGF-1 receptors in the normal spinal cord as well as the change of these receptors in diseased spinal cord may be of importance in future treatment strategies of ALS.

Index Entries: Amyotrophic lateral sclerosis; human; insulin-like growth factors; insulin-like growth factor receptors; nerve growth factor receptors; motor neurons, receptor autoradiography; spinal cord.

Introduction

Amyotrophic lateral sclerosis, the most common of motor-neuron diseases, which begins in middle or late life, is a devastating neurologic disease that affects specific populations of neurons, i.e., lower motor neurons in brain stem and spinal cord and upper motor neurons in neocortex. In the spinal cord, a severe degeneration of ventral horn motor neurons is observed. Striated muscles show features of degeneration and collateral reinnervation that result in the formation of motor units with abnormally large size. By definition, the features of ALS are signs and symptoms of lower motor neuron dysfunction, including focal and multifocal weakness, atrophy, cramps, and fasciculation associated with the corticospinal tract signs of spasticity, and enhanced and pathologic reflexes *(1,2)*. In the majority of cases, ALS takes a relentlessly progressive, downhill course over a period of 1–5 yr *(3,4)*. The disease occurs in both sporadic and familial forms. Familial ALS constitutes about 5–10% of all

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cases. It is proposed to be inherited as an autosomally dominant disorder with low penetrance. The lesions in familial ALS seem in most aspects to be identical with the sporadic form of ALS. More than a century after the first characterization of the disease by Charcot and Joffroy *(5)*, the underlying etiological mechanisms are still poorly understood. Research strategies to understand the etiology of ALS have included the search for viruses, neurotoxic factors, autoimmunity, and limitations or reductions of trophic factors necessary for the survival of motor neurons *(6,7)*.

Recently, Rosen and coworkers *(8)* reported that the disorder is tightly linked to the Cu/Zn-superoxide dismutase gene (SOD 1) on chromosome 21 in some, but not all, familial ALS pedigrees. However, the functional importance of these missense mutations remains to be explored. It also remains to be determined why these SOD 1 mutations specifically affect motor neurons. Nevertheless, these results may offer insight into the causes of familial ALS, which is not linked to SOD 1 mutations, and of sporadic ALS, as well as provide leads for new therapeutic strategies. With regard to the neurotrophic hypothesis, several possibilities might pertain to the pathogenesis of ALS; these include a failure in neurotrophic hormone release by muscle, a failure of uptake of the factor by the presynaptic motor axon terminal, and impairment in the retrograde axonal transport of the factor to the cell body *(9)*. Some growth factors, such as the insulin-like growth factors and neurotrophins, have received special attention with regard to their putative role in the regulation of motor-neuron maintenance and/or survival.

Nerve growth factor (NGF) is a 229-amino-acid protein that is expressed in a variety of peripheral tissues as well as in the brain. NGF is a member of a family of structurally related peptide factors called neurotrophins. It is required for the survival and differentiation of sympathetic and some sensory neurons in the peripheral nervous system, and of certain populations of cholinergic neurons in the central nervous system *(10,11)*. Binding studies using radiolabeled NGF have revealed a low-affinity (LNGFR) and a high-affinity (HNGFR) binding site *(12,13)*. The low-affinity NGF receptor (LNGFR) is a 75-kDa glycoprotein that lacks signal-transmitting motif, such as a protein kinase *(14)*. The high-affinity receptor, on the other hand, has been shown to be a receptor protein distinct from the LNGFR *(14)* and is likely to be identical with the trk proto-oncogene. The low affinity receptor is reported to be expressed in neurons as well as in Schwann cells *(15)*, whereas the high-affinity receptor is only found in neurons.

The insulin-like growth factors (IGFs) have been proposed as growth and maintenance hormones within the human brain *(16)*. In mammals, in addition to being found in the circulation, IGFs are also found in the central nervous system *(17–19)*. IGF-1 receptors have been demonstrated to be present in the rat *(19,20)* and human central nervous system *(21,22)*. More recently, these receptors have been localized more directly to specific human brain regions using autoradiographic techniques *(23)*. In the present study, the localization of NGF and IGF-1 receptors in human spinal cords from controls and ALS cases was studied using quantitative receptor autoradiography.

Materials and Methods

Postmortem Tissue

Spinal cords from four ALS-cases (mean age ± SEM = 76 ± 1 yr) and four age-matched controls (mean age ± SEM = 75 ± 3 yr) without any documented CNS lesions were obtained at autopsy performed at the University Hospital of Uppsala. The main cause of death in the case of controls was circulatory insufficiency. The mean postmortem time for ALS cases and controls was 21 ± 5 and 30 ± 4 hr (mean ± SEM), respectively. ALS was diagnosed based on clinical examinations and electromyography. The mean duration of the disease in these cases was 3 ± 2 yr. At autopsy the spinal cords were taken out and immediately sectioned into 3–5 mm slices that were frozen between metal plates in liquid nitrogen and sealed in small plastic boxes that were stored at –70°C.

Autoradiography and Image Analysis

For autoradiography, 10-μm cryosections were cut from the different spinal segments with a Reichert and Jung (Germany) cryomicrotome and thaw-mounted on chrome-alum gelatin-coated slides, which were then put in a dessicator to dry for at least 4 h followed by a few days of storage in a dessicator at –20°C. IGF-1 receptor autoradiography was performed as described previously *(23)*. Briefly, sections were preincubated at +4°C in 50 m*M* Tris-HCl buffer (pH = 7.7) for 10 min. The sections were then incubated with 30 p*M* recombi-

nant ^{125}I-insulin-like growth factor 1 (^{125}I-IGF-1, Pharmacia, Sweden) in Tris-HCl buffer for 20 h at +4°C. Adjacent sections were incubated in the presence of 0.1 μ*M* IGF-1 to obtain nonspecific binding.

NGF receptor autoradiography was performed as described by Richardson et al *(24)*. Briefly, sections were preincubated at 20°C for 2 × 15 min in a 0.1*M* PBS buffer, at pH 7.4, containing 0.5 m*M* $MgCl_2$, cytochrome C (1 mg/mL, leupeptine) (0.4 μg/mL) and 0.5 m*M* phenyl methyl sulfonic fluoride (PMSF). Sections were then incubated for 90 min at 20°C in the same buffer system with the addition of 60 p*M* ^{125}I-NGF (Amersham, UK). Four nonspecific binding sections were incubated with 60 n*M* nonlabeled murine β-NGF in the above described ^{125}I-NGF solution. After incubation, the sections were rinsed in their respective buffer, dipped in distilled water to remove buffer salts, dried using a stream of cold dry air, and placed against tritium-sensitive film (Hyperfilm, Amersham, UK) for 2 wk at –20°C or 4 d at 4°C in the case of IGF-1 and NGF, respectively. Calibrated radioactive "standards" (Amersham) were exposed and developed in parallel with tissue sections to permit conversion of mean gray densities to molar concentrations of receptor bound radioligand. In both IGF-1 and NGF binding, specific binding was obtained by subtracting the nonspecific binding from the total binding. The quantitative analysis of the autoradiograms was performed using computer-assisted image analysis (Imteck, Uppsala, Sweden). Statistical analysis of data was performed by one-way ANOVA.

Histopathology

To quantify the neuronal loss in the ALS material under study, somatic motor neurons were counted in 10-μm spinal cryosections. The total number of large ventral horn neurons was quantified in sections stained with thionine representing the different spinal segments. Each region was counted bilaterally and in two consecutive sections.

Results

The distribution of IGF-1 and NGF binding sites in human spinal cords from controls and ALS patients was studied by quantitative receptor autoradiography. Nonspecific binding (in the presence of unlabeled IGF-1 or NGF) did not exceed 10 and 30% of total binding for IGF-1 and NGF, respectively (Fig. 1). Quantitative measurement of ^{125}I-IGF-1 autoradiography in the different segmental levels of the normal human spinal cord revealed high concentrations of IGF-1 binding sites in the central canal and the dorsal horn, whereas moderate levels were observed in lamina X, intermediate zone, and ventral horn (Table 1). Low levels of specific ^{125}I-IGF-1 binding were detected in the dorsal, lateral, and ventral white matter. Moreover, the lowest binding was consistently obtained in the dorsal white matter area in the different segmental levels of the spinal cord. In the ALS cases, although a greater concentration of IGF-1 binding sites was observed in all spinal segments, a significant increase in binding was observed in the cervical and sacral segments (Table 1). The number of these sites was significantly increased in the ventral horn, an area that shows extensive neuronal degeneration in ALS and intermediate zone (laminae III–VIII), as well as in the dorsal, lateral, and ventral white matter in the cervical spinal segments. However, in the case of the sacral segments, the increase in ^{125}I-IGF-1 binding was observed in the intermediate zone (laminae III–VIII) and lamina X (Table 1). Interestingly, these results indicate that the intermediate zone (laminae III–VIII), which consists mainly of interneurons, is affected in ALS.

Table 2 presents the HNGFR concentrations obtained by quantitative autoradiographic determinations. Specific binding was predominantly located in the dorsal horn of the spinal cord, most likely within the superficial layers (Rexed laminae I and II) (Table 2). No ^{125}I-NGF binding could be detected in the ventral horn region or in myelinated regions. High concentrations (0.5–1.0 fmol/mg wet wt) of HNGFR were found in the substantia gelatinosa of the dorsal horn of the spinal cord. The highest levels of binding sites were observed in the dorsal horn of the sacral cord (Table 2). However, the distribution pattern and concentration range of HNGFR was similar at the cervical, thoracic, and lumbar levels. The autoradiographic data suggest that there is no alteration of high-affinity ^{125}I-NGF binding sites in any of the analyzed regions of the spinal cord in ALS (Table 2). Moreover, specific binding was not observed in the ventral horn in any of the analyzed sections.

The number of motor neurons in the ventral horn of sections from controls and ALS patients showed significant reductions in the number of motor neurons in the ventral horns of cervical and lumbar spinal segments from ALS patients. All levels of the

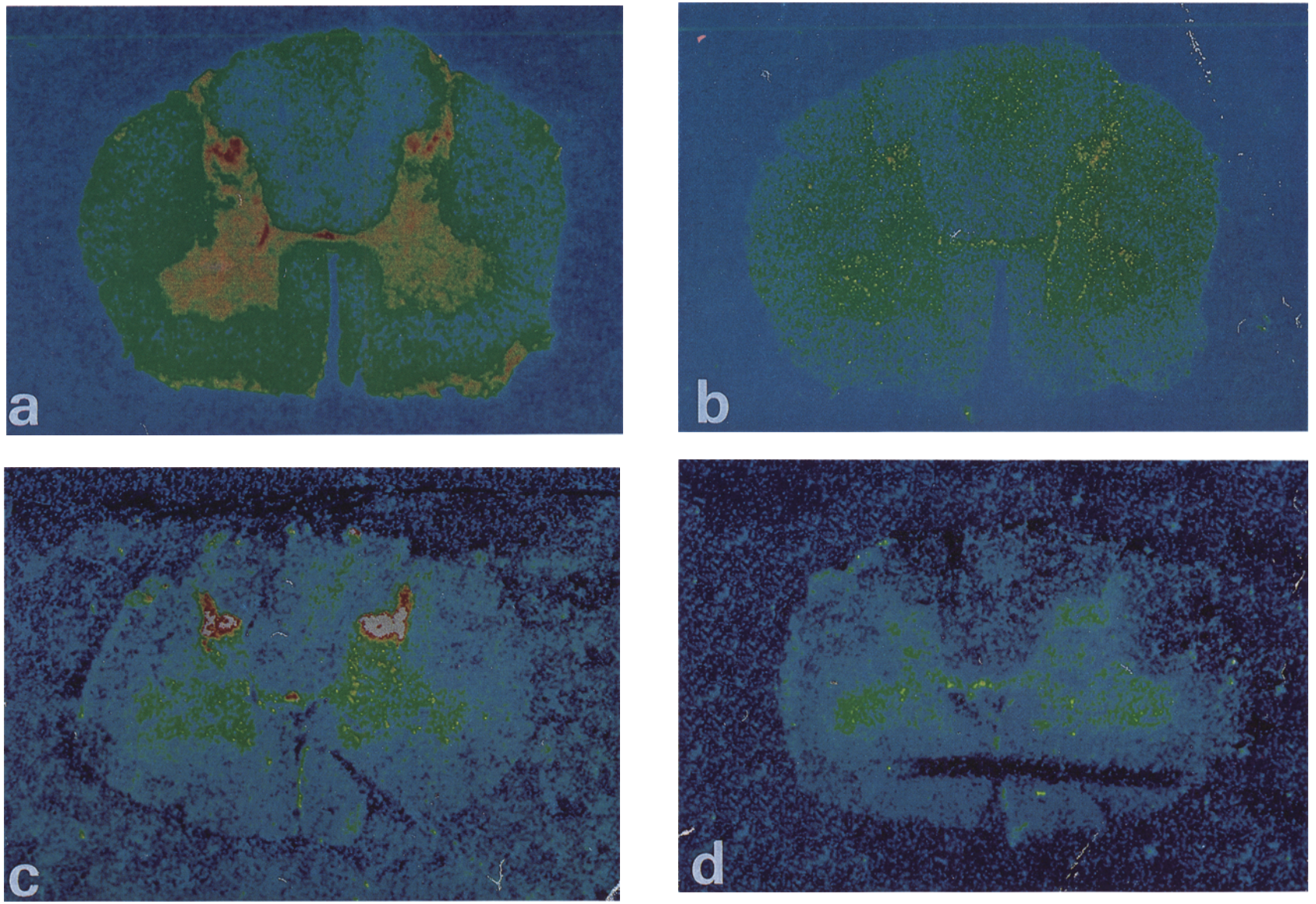

Fig. 1. ^{125}I-IGF-1 **(A, B)** and ^{125}I-NGF **(C, D)** binding in the spinal cord. Figures are representative of total ^{125}I-IGF-1 binding in the absence of excess unlabeled IGF-1 (A) and nonspecific ^{125}I-IGF-1 binding in the presence of excess unlabeled IGF-1 (B), and total ^{125}I-NGF binding in the absence of excess unlabeled NGF (C) and nonspecific ^{125}I-NGF binding in the presence of excess unlabeled NGF (D).

Table 1
Specific IGF-1 Receptor Binding in Different Regions of Spinal Segments from Control and ALS Human Spinal Cord

Spinal level	Specific bound ^{125}I-IGF-1 pmol/g wet wt ± SEM	
	Control	ALS
Cervical		
Dorsal horn	0.83 ± 0.07[b]	0.96 ± 0.07[b]
Intermediate	0.41 ± 0.03	0.65 ± 0.06[d]
Lamina X	0.48 ± 0.08	0.72 ± 0.09
Central canal	1.20 ± 0.25	1.63 ± 0.33
Ventral horn	0.32 ± 0.02	0.66 ± 0.08[d]
Dorsal white matter	0.08 ± 0.02	0.20 ± 0.03[c]
Lateral white matter	0.10 ± 0.02	0.29 ± 0.05[d]
Ventral white matter	0.11 ± 0.01	0.30 ± 0.06[c]
Thoracal		
Dorsal horn	0.59 ± 0.05[b]	0.73 ± 0.10
Intermediate	0.35 ± 0.04	0.44 ± 0.06
Lamina X	0.37 ± 0.06	0.54 ± 0.11
Central canal	1.16 ± 0.32	1.45 ± 0.32
Ventral horn	0.29 ± 0.04	0.47 ± 0.12
Dorsal white matter	0.09 ± 0.01	0.13 ± 0.03
Lateral white matter	0.11 ± 0.02	0.19 ± 0.05
Ventral white matter	0.12 ± 0.03	0.24 ± 0.07
Lumbar		
Dorsal horn	0.68 ± 0.10	0.94 ± 0.13
Intermediate	0.26 ± 0.03	0.50 ± 0.12
Lamina X	0.30 ± 0.03	0.56 ± 0.16
Central canal	0.61 ± 0.09	1.12 ± 0.31
Ventral horn	0.24 ± 0.02	0.42 ± 0.12
Dorsal white matter	0.07 ± 0.01	0.14 ± 0.03
Lateral white matter	0.11 ± 0.01	0.23 ± 0.07
Ventral white matter	0.11 ± 0.02	0.20 ± 0.06
Sacral		
Dorsal horn	0.60 ± 0.13	1.12 ± 0.20
Intermediate	0.26 ± 0.04	0.76 ± 0.20[c]
Lamina X	0.33 ± 0.06	0.85 ± 0.20[c]
Central canal	1.19 ± 0.07	1.60 ± 0.47
Ventral horn	0.31 ± 0.08	0.60 ± 0.15
Dorsal white matter	0.11 ± 0.02	0.21 ± 0.05
Lateral white matter	0.21 ± 0.02	0.36 ± 0.08
Ventral white matter	0.17 ± 0.03	0.32 ± 0.10

[a]Values are mean ± SEM (n = 4).
[b]Mean ± SEM (n = 3).
[c]$p < 0.05$.
[d]$p < 0.01$ significantly different from control.

ALS cases studied showed a 40–60% numerical reduction of motor neurons (Table 3).

Discussion

ALS is characterized by a preferential, progressive degeneration of upper and lower motor neurons. The underlying etiological mechanisms of the disease are not yet known. The neuronal degeneration seen in ALS has been suggested to be the result of loss of neurotrophins in the spinal cord (*9*). The loss of excess neurons during development is regulated via trophic factors that are produced in limited quantities and that support distinct neuronal

Table 2
^{125}I-NGF Binding in Cryosections of Human Spinal Cord as Estimated with a Quantitative Autoradiographic Method

	Specific bound ^{125}I-NGF, fmol/mg wet wt	
Spinal levels	Controls	ALS cases
Cervical	0.42 ± 0.09	0.33 ±0.11
Thoracic	0.28 ± 0.05	0.22 ±0.12
Lumbar	0.44 ± 0.07	0.48 ±0.06
Sacral	0.59 ± 0.16	0.48 ±0.11

[a]Values are given as fmol/mg wet weight ± SEM.

Table 3
Number of Motor Neurons in the Ventral Horn[a]

Spinal levels	Controls	ALS cases
Cervical	55 ± 8.20	27 ± 2.68[b]
Thoracic	17 ± 2.52	11 ± 4.35
Lumbar	68 ± 12.17	22 ± 5.72[c]
Sacral	45 ± 6.23	25 ± 5.36

[a]Values are mean ± SEM (n = 4).
[b]$p < 0.05$.
[c]$p < 0.02$ significantly different from controls (Student's unpaired t-test).

populations. Thus, some of these factors are needed for neuronal survival and maintenance in the adult nervous system. Moreover, neurotrophic factors are most likely involved in regeneration and maintenance of neuronal integrity after injury. In the present study, quantitative autoradiography was applied to investigate the regional distribution of IGF-1 and NGF receptors in the normal human spinal cord. Moreover, we report the distribution of IGF-1 and NGF receptors in spinal cords from ALS patients.

The quantitative autoradiographic receptor studies demonstrated the same distribution pattern of IGF-1 receptors in the different spinal levels of normal spinal cord, with the highest concentration in the central canal > dorsal horn > ventral horn > white matter. In contrast to the central canal, the white matter areas showed low IGF- 1 receptor concentration (Table 1). The dorsal white matter area showed the lowest IGF-1 receptor concentration, indicating that the somatosensory fiber tracts to the brain do not posses abundant IGF-1 receptors. In the ALS cases, a general upregulation of IGF-1 receptors was observed throughout the spinal cord. The greatest increase was observed in the sacral and cervical segments in ALS. Interestingly, the increase in IGF-1 receptors in the ventral horn of the cervical segment is in contrast to the decrease of motor neurons in that area, indicating that the increased number of receptors in ALS may not be localized on motor neurons. Recently, Aquilonius and coworkers *(25)* reported a significant increase of glia cells in the ventral horn of ALS cases. It is most likely that the high number of IGF- 1 receptors seen in ALS spinal cord are located on glial cells whose proliferation is induced in ALS. However, a strong upregulation of these receptors within the remaining motor neurons cannot be excluded.

NGF has been shown to play an important role in the differentiation and growth of sympathetic and sensory neurons *(10,26)*. Another line of evidence implicates a trophic action on neurons of the basal forebrain *(27,28)* and the caudate-putamen *(29)*. It is now presumed that NGF is secreted from neuronal target tissue and that it mainly reaches the neuronal soma by retrograde transport *(30)*. The functional importance of NGF within the spinal and bulbar motor neuron pools is less well understood. In animal experiments, crush lesions of the sciatic nerve are accompanied by a strong LNGFR reexpression in motor neurons, with maximal mRNA expression and immunohistochemical detectability approx 7 d after lesion and subsequent decline to the normal zero level after about a month *(31,32)*. However, after other types of mechanical lesions, such as axotomy, that lead to neuronal death, receptor reexpression is either very slight or nonexistent *(32)*. Furthermore, NGF was shown to have no effect on the survival of axotomized motor neurons in newborn rats. From these animal studies, it could be argued that the reexpression of NGFR is a limited temporal event and that only motor neurons affected to a certain degree would exhibit this reexpression. It has been shown by immunohistochemical techniques that LNGFR was not reexpressed in motor neurons in ALS *(33)*. Moreover, in this study, no reexpression of high-affinity NGF receptors could be demonstrated in the motorneuron and other areas of the spinal cord of ALS patients despite the survival of 40–60% of the motor neurons at all cord levels. Thus, it may be concluded that NGF, which has trophic effects in neurons, seems not to have a role in ALS. The present demonstration of an enhancement of IGF-1 receptors in the ALS spinal cord indicates that IGF-1 may not be involved in the etiology of the disease. However, the increase in IGF-1 receptor is likely to constitute

part of the CNS repair response to degeneration. IGFs and the IGF-1 receptor have earlier been implicated in the CNS response to injury induced by lesions *(34)*, cerebral ischemia *(35)*, and demyelination *(36)*. The IGFs are potent inducers of collateral sprouting when injected into skeletal muscle *(37)* and are shown to be retrogradely transported in spinal motor neurons. Moreover, Kanje and coworkers *(38)* showed that endogenous extracellular IGF-1 plays an important role during regeneration of peripheral nerve fibers. Thus, we suggest the application of IGF-1 may prove beneficial in the future treatment of ALS.

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