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Spinal cord mGlu1a receptors
Possible target for amyotrophic lateral sclerosis therapy

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of motor neurons, whose pathogenesis, probably multifactorial, is thought to involve AMPA/kainate receptor-mediated Ca^{2+} influx and excitotoxicity. We evaluated the possible involvement of Group I metabotropic glutamate (mGlu) receptors in the control of motor neuron viability. mGlu1a receptor distribution was analyzed in rat and human spinal cord by immunohistochemistry. In both species, the expression of mGlu1a receptor was developmentally regulated and showed a general trend to increase during foetal and postnatal maturation, reaching the maximum level of expression in the dorsal laminae I–II and in motor neurons in adult life. Exposure of spinal cord slices from adult rats to 300 μM kainate for 30 min induced motor neuron death, which was prevented by the Group I mGlu receptor agonist 3-hydroxyphenylglycine (3-HPG; 100 μM). Since motor neurons do not express mGlu5 receptors, mGlu1a receptor activation might be responsible for the observed neuroprotection. mGlu1a immunohistochemistry was conducted on spinal cord autoptic specimens from ALS and control subjects. Surviving motor neurons from ALS spinal cord still revealed the presence of mGlu1a at levels comparable to that from controls. We suggest that mGlu1a receptors may act as suitable targets for ALS experimental therapies. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Metabotropic glutamate receptor; Spinal cord; Development; Rat; Human; Motor neurons; Amyotrophic lateral sclerosis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disease, with a prevalence of ~5 per 100,000 individuals, characterized by the selective degeneration of motor neurons. In patients with ALS, the symptoms are primarily those of weakness, which may start in the hands or legs or be manifested by slurred speech and dysphagia. On examination, there are almost always both lower and upper motor neuron signs. The loss of motor neurons leads to a progressive atrophy of skeletal muscle and, ultimately, death due to respiratory failure in the majority of ALS patients within 2–5 years of clinical onset. Approximately 10% of ALS patients are familial cases, while the majority of ALS cases (90%) are sporadic with no known genetic component.

Current research evidence suggests that genetic factors, oxidative stress and glutamatergic toxicity, with damage to critical target proteins and organelles, may be important contributory factors to motor neuron injury in ALS (Julien, 2001). Particular emphasis has been given to excitotoxic hypothesis (Shaw and Ince, 1997), due to a dysfuction of glutamate uptake in the ALS spinal cord (Rothstein, 1995a,b). Decreased levels of the astroglial glutamate transporter EAAT2 have been found in motor cortex and spinal cord from ALS patients, which are due at least in part to abundance of aberrant EAAT2 mRNA species resulting from RNA processing errors (Lin et al., 1998). High synaptic levels of glutamate can cause an inappropriate

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activation of neuronal glutamate receptors which results in cell death via alterations in cytosolic Ca^{2+} homeostasis. Riluzole, a rather unspecific glutamate antagonist, is the only drug approved by the Food and Drug Administration for the treatment of ALS, but the drug showed only transient and marginal benefits in clinical trials (see Rowland and Shneider, 2001, for review). It is coinceivable that different, novel approaches toward a safe and specific modulation of glutamatergic neurotransmission could contribute to the process of identifying potential ALS therapies.

Glutamate, the chief excitatory neurotransmitter in the central nervous system, acts through two classes of receptors: the glutamate-gated ion channels and G-protein-coupled receptors. The ionotropic group includes the AMPA/kainate receptors which seem to determine the progressive loss of motor neurons in ALS (Williams et al., 1997). On the other hand, metabotropic glutamate (mGlu) receptors include eight subtypes that can be divided into three groups according to their amino acid sequences, coupling to signal transduction pathways and agonist sensitivity (Conn and Pin, 1997; Schoepp et al., 1999). Group I mGlu receptors (mGlu1 and mGlu5 subtypes) activate protein kinase C (PKC) through IP_3-mediated triggering of Ca^{2+}-release from intracellular stores. Alternate splicing of pre-RNA for Group I mGlu receptors generate multiple receptor isoforms: mGlu1a, b, c, d, f and mGlu5a and b (Joly et al., 1995), which display various degrees of carboxy-terminal protein truncations, resulting in cytoplasmic tails of variable length which can affect the coupling efficiency to GTP-binding proteins. Several reports from our group (Pizzi et al., 1996) as well as others (Nicoletti et al., 1996) indicate that mGlu receptor agonists are endowed with neuroprotective properties against excitotoxic insults, suggesting that mGlu receptors could be suitable targets for novel pharmacological strategies in the treatment of neurodegenerative diseases. While Groups II and III mGlu receptor agonists are consistently neuroprotective, results with Group I mGlu receptor agonists have been mixed, showing either amplified or attenuated excitotoxicity with different experimental conditions (Nicoletti et al., 1999). We recently reported that mGlu agonists, including 3-hydroxyphenylglycine (3-HPG), a Group I selective agonists, consistently inhibit kainate-induced excitotoxicity of motor neurons in rat spinal cord slices (Pizzi et al., 2000).

We previously assessed the expression of the individual mRNAs encoding for the different mGlu receptor subtypes in rat spinal cord (Valerio et al., 1997a). The results indicated a selective expression of mGlu1a versus the 1b variant, while in contrast both the a and b isoforms of the mGlu5 receptor were clearly evident. A recent in situ hybridization has provided further information on the relative expression of the Group I mGlu receptors in the rat spinal cord (Berthele et al., 1999). Thus, the mGlu1 receptor displays a broad distribution in the spinal grey matter with a selective expression of mGlu1a splice variant in motor neurons. In contrast, the mRNA for mGlu5 receptor is highly expressed in dorsal horns and is absent in motor neurons, as already demonstrated by immunocytochemistry studies conducted both in rat (Vidnyanszky et al., 1994) and in human spinal cord (Valerio et al., 1997b).

The present study focused on spinal cord mGlu1a receptor; in particular, we investigated on the time of appearance and the anatomical localization of mGlu1a receptor protein in the human spinal cord during foetal development and in adult life. Data were compared with those obtained by the analysis of spinal cord from embryonal (E15), neonatal (P8) and adult rats. Moreover, we evaluated the possible changes in the expression of mGlu1a receptor protein in spinal cord autoptic specimens from ALS patients.

2. Materials and methods

2.1. Immunohistochemistry

Adult human spinal cords (cervical, thoracic and lumbar levels) used for the study of the localization of mGlu1a receptors were obtained at autopsy from male subjects (35 and 41 years) who died of non-neurological diseases from the Brain Bank, Department of Neurological and Visual Sciences, Verona University, Italy. Samples were dissected 24 h after death and immediately fixed by immersion in 4% paraformaldehyde in 0.1% phosphate buffer for 12 h. Human spinal cords used for the comparison of control versus diseased tissue (cervical and thoracic levels) were obtained at autopsy from six sporadic ALS patients (mean age, 60.1 ± 8.3 years) and four control subjects who died of non-neurological diseases from the Brain Bank, The Maudsley Institute of Psychiatry, London, UK. Postmortem interval was below 24 h in all these cases. Details of ALS and control subjects used in the study are outlined in Table 1. These tissues were paraffin wax embedded and sectioned at 7 μm. Foetal human spinal cord tissue were obtained from legally aborted foetuses. All procedures were in accordance with current Italians laws. The gestational age of each specimen was checked by comparison of the spinal cord structure with that reported previously (Hamilton and Mosmann, 1972).

Table 1
Summary of clinical data of ALS patients and controls

sALS cases	Sex	Age	Cause of death
A81/96	M	64	bronchopneumonia
A101/96	F	46	respiratory failure
A138/96	F	66	bronchopneumonia
A160/96	F	66	motor neurone disease
A193/96	M	54	pneumonia
A281/96	F	65	motor neurone disease
Control cases			
A264	F	86	peritonitis
A38/95	M	76	bronchopneumonia
A224/96	F	64	cancer bladder
A196/96	M	86	myocardial infarction

Spinal cords and dorsal root ganglia (DRG) from E15 rat embryos and P8 neonatal rats of Sprague–Dawley strain (Charles River) were dissected and fixed by immersion in 4% paraformaldeyde in 0.1 M phosphate buffer for 12 h. Spinal cords and DRG from adult rats were dissected after transcardial perfusion with 4% paraformaldeyde in 0.1 M phosphate. All tissues were then cryoprotected through graded sucrose concentration (5–30%) at 12 h interval. Immunohistochemistry was conducted as previously described (Valerio et al., 1997b). Briefly, lumbar spinal cord and DRG sections (10 μm) were pretreated with 1% hydrogen peroxide in PBS and incubated overnight at 4 °C in mGlu1a antiserum (Chemicon International, Tamecula, CA) diluted in PBS containing 5% nonfat dried milk and 0.2% triton X-100. Immunoreactivity was visualized with the biotin/avidin system (Vectastain ABC kits, Vector, Burlingame, CA) followed by reaction with 3′,3′-diaminobenzidine (Sigma-Aldrich, St. Louis, MO). Image analysis was performed using the Analytical Imaging Station (AIS) Software (Imaging Research). Integrated optical density (IOD) measurements represent the mean density value of all the pixels in the cell of interest. The relative optical density (ROD) is an inverse logarithmic function of grey level values.

2.2. Motor neuron toxicity

Male 40-day-old Sprague–Dawley rats (180 g) were anaesthetized with ether and decapitated according to Policy on the Use of Animals in Neuroscience Research. Experiments were carried out on transverse slices of lumbar spinal cord at a thickness of 0.7 mm, as described (Pizzi et al., 2000). Briefly, slices were submerged in Krebs solution (120 mM NaCl, 2 mM KCl, 26 mM $NaHCO_3$, 1.9 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11 mM glucose, 2 mM $CaCl_2$, pH 7.4) and preincubated at 37 °C for 30 min under O_2–CO_2 conditions, then drugs were added and incubation carried out for 30 min. Slices were then washed and further incubated in fresh buffer for 60 min to allow reversibly damaged cells to recover. Slices were then fixed and embedded in paraffin. Sections were cut at 5 μm thickness, stained with methylene blue and azur II and examined by light microscopy. The percentage of cell survival was calculated as the ratio of the number of survived motor neurons to the number of total motor neurons. In each experiment, at least five slices from different spinal cords were subject to each conditions. Statistical analysis was performed by Kruskal–Wallis nonparametric ANOVA with adjustments for multiple comparisons.

3. Results

3.1. Immunohistochemical localization of mGlu1a receptor in rat spinal cord

Immunohistochemistry was performed to identify mGlu1a receptor distribution in rat spinal cord tissue at various developmental stages and during adult life. Since the staining pattern of mGlu1a immunoreactivity was similar throughout the rostrocaudal extent of the spinal cord, only data regarding the lumbar region are reported in the present study. The age-related changes of mGlu1a receptor distribution in rat spinal cord are summarized in Table 2.

At early stage of rat foetal life (E15) we detected mGlu1a receptor immunostaining mainly in the dorsal horns, where the receptor was expressed at very low levels (Fig. 1A). Spinal cords from P8 rats showed an increase of the immunoreactivity in the dorsal horns (Fig. 1B); moreover, at this developmental stage, a low but detectable level of immunostaining also appeared in DRG cells with heterogeneous distribution, being discretely localized to smaller neurons (not shown). A pronunced mGlu1a receptor labeling was found in adult rat spinal cord. mGlu1a receptor appeared to be expressed in moderate amount in the superficial laminae (*Rexed* I and II) of the dorsal horns (Fig. 1C). Furthermore, a dense immunoreactivity clearly appeared in the ventral horns, particularly motor neurons somata and axons resulted to be highly mGlu1a receptor-immunoreactive (Fig. 1C). The pattern of mGlu1a receptor immunolabeling in adult DRG consisted of a heterogeneous staining as already found in rat P8 specimens, although adults exhibited a level of immunoreactivity stronger than that observed in neonatal tissue. In particular, small (<35 μm) or medium (35–60 μm) diameter neurons appeared to be intensely immunoreactive to anti-mGlu1a antibody. Unlabeled neurons appeared predominantly of large (>60 μm) size (not shown).

3.2. Immunohistochemical localization of mGlu1a receptor in human spinal cord

Examination of the mGlu1a receptor immunoreactivity was carried out on transverse sections along the whole cervical–lumbar extension of foetal and adult human spinal cord. Since no significant differences in the staining pattern

Table 2
Summary of the localization of mGluR1a immunoreactivity during the development of rat spinal cord and dorsal root ganglion

Region	Foetal (E15)	Neonatal (P8)	Adult
Dorsal horn	+	++	++[a]
Ventral horn	±	±	+++[b]
Dorsal root ganglion[c]	±	+	++

−, no immunoreactivity; ±, questionable immunoreactivity; +, sparse immunoreactivity; ++, moderately dense immunoreactivity; +++, highly dense immunoreactivity. nt, not tested.

[a] Laminae I and II.

[b] Motor neurons.

[c] Heterogeneous staining.

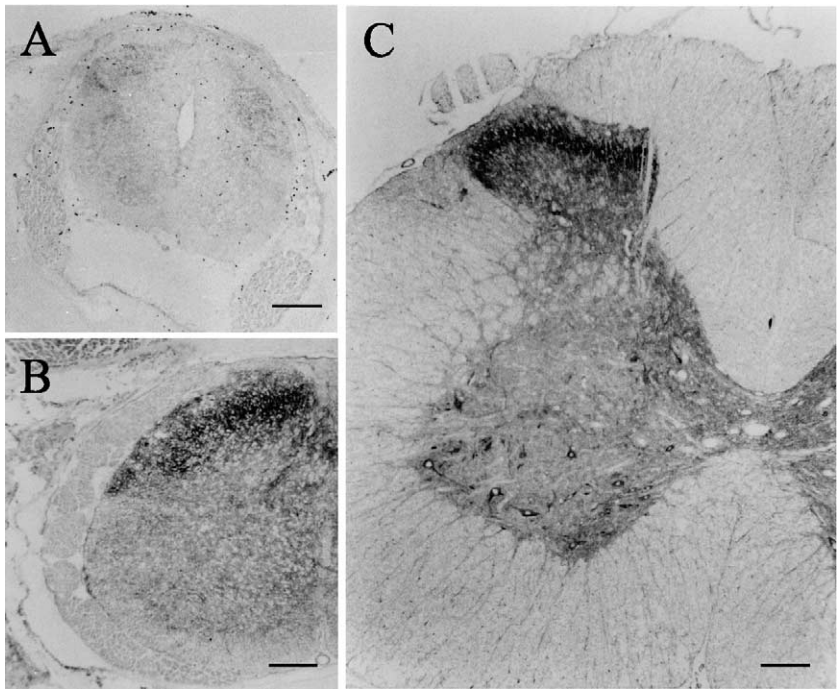

Fig. 1. Low power microphotographs showing the changes in mGlu1a receptor immunoreactivity in transverse rat spinal cord sections (lumbar level) during development. (A) E15 rat embryo. (B) P8 neonatal rat. (C) Adult rat (all scale bars, 200 μm).

at different spinal cord levels were found, only data regarding the lumbar sections are summarized in Table 3.

We found no appreciable mGlu1a staining in the developing spinal cord horns at 6 weeks of gestation, with the exception of a moderately dense immunoreactivity in the ventral marginal layer (Fig. 2A). A sparse immunoreactivity was first observed in dorsal and ventral horns of a 10-week-old human foetus (not shown). A stronger expression of the mGlu1a receptor in human spinal cord was observed starting from the twelth week of gestation. Both dorsal and ventral horns showed a moderately dense immunoreactivity, moreover, a pale staining appeared heterogeneously in DRG cells: the intensity of the labeling varied among individual neurons, so that weakly stained perycaria were found together with moderately stained perycaria (Fig. 2B). A similar labeling pattern was evident in a foetal specimen at the twenty-second gestational week. In particular, at this developmental stage, an intense immunostaining was detected in motor neurons of the ventral horns (not shown). By analyzing the pattern of mGlu1a receptor immunolocalization in adult human spinal cord, we found a moderately dense immunoreactivity in the superficial laminae (*Rexed* I–II) of the dorsal horns (Fig. 2C); moreover, a very intense label was present in the somata of ventral horn motor neurons (Fig 2D).

Table 3
Summary of the localization of mGluR1a immunoreactivity in foetal (6–22 weeks of gestation) and adult human spinal cord and dorsal root ganglion

Region	6 weeks	10 weeks	12 weeks	22 weeks	Adult
Dorsal horn	–	+	++	++	++[a]
Ventral horn	±	+	++	+++[b]	+++[b]
Dorsal root ganglion[c]	±	nt	+	nt	nt

–, no immunoreactivity; ±, questionable immunoreactivity; +, sparse immunoreactivity; ++, moderately dense immunoreactivity; +++, highly dense immunoreactivity. nt, not tested. A moderately dense immunoreactivity was also observed in the ventral marginal layer at 6 weeks of gestation. No immunoreactivity was detected in the white matter nor in the dorsal or ventral roots.

[a] Laminae I and II.

[b] Motor neurons.

[c] Heterogeneous staining.

3.3. Neuroprotective effect of Group I mGlu receptors on kainate-induced motor neuron degeneration

We applied an in vitro model of kainate-dependent neurotoxicity to investigate the possible role of Group I mGlu receptors in the control of motor neuron vulnerability. As more extensively described (Pizzi et al., 2000), 30 min exposure of lumbar spinal cord slices from adult rats to 300 μM kainate reduced motor neuron survival to 32% of control value (Table 3). Kainate toxicity was completely prevented by adding to the culture medium the Group I/II mGlu receptor agonist (1*S*,3*R*)-ACPD at a concentration of 50 μM. The neuroprotective effect of (1*S*,3*R*)-ACPD was reversed by the mGlu receptor blocker (+)-α-methyl-4-carboxyphenyl-glicine (MCPG) at 500 μM concentration. In order to evaluate the contribution of Group I mGlu receptors to neuroprotection, we used the selective Group I agonist, 3-hydroxyphenylglycine (3-HPG). By exposing

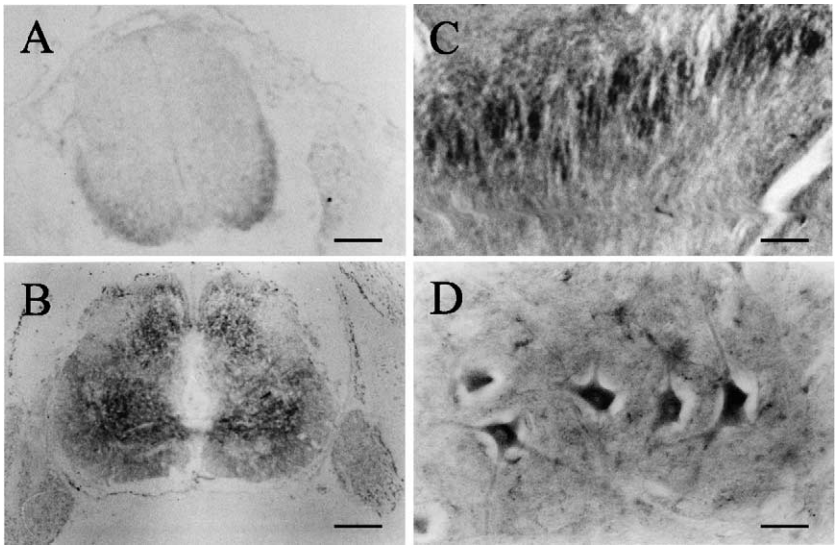

Fig. 2. Low power microphotographs showing the pattern of mGlu1a receptor immunoreactivity in transverse spinal cord sections (lumbar level) of human foetuses at 6 (A) and 12 (B) gestational weeks. Scale bars, 300 μm. Micrographs showing a section of an adult human spinal cord at the level of the lumbar enlargement. mGlu1a immunoreactivity is found in the superficial layers of the dorsal horns (C) and in motor neurons (D). Scale bars, 200 μm (C) and 100 μm (D).

spinal cord slices to 100 μM 3-HPG during kainate treatment, we observed a significant increase in motor neuron viability to 87% survival of control (Table 4).

3.4. mGlu1a receptor immunohistochemistry in human spinal cord from control and ALS patients

mGlu1a receptor immunohistochemistry was carried out on autoptic specimens (cervical or thoracic segments) from spinal cord of six sporadic ALS patients and four control cases. In all the examined specimens from human control cases, the overall pattern of mGlu1a immunoreactivity was superimposable to that already described (Fig. 2C,D and Table 3). In particular, normal motor neurons were strongly labelled by mGlu1a antiserum (Fig. 3A). Spinal cord tissue from ALS patients appeared dramatically degenerated: motor neuron somata became shrunk and atrophic, they lost their typical multipolar morphology and acquired a spindle-shaped aspect with a diameter of 20–30% smaller than

Table 4
Kainate-mediated motor neuron death in rat spinal cord slices is prevented by stimulation of Group I mGlu receptors

Treatment	Motor neuron survival (%)
Control	97±3
KA 300 μM	32±7*
KA+(1*S*,3*R*)-ACPD 50 μM	98±2
KA+(1*S*,3*R*)-ACPD+MCPG 500 μM	49±3*
KA+3-HPG 100 μM	87±12

Test drugs were added to the slices 5 min before kainate (KA) at the indicated concentrations, then KA was added and incubation was carried out for another 30 min. At the end of incubation, slices were left to recover in fresh buffer for additional 60 min. Values represent the means±S.E.M. of three experiments run in quintuplicate. Statistical significance of the differences was analysed by Kruskal–Wallis nonparametric ANOVA with adjustments for multiple comparisons.

* $P<.01$ versus control value.

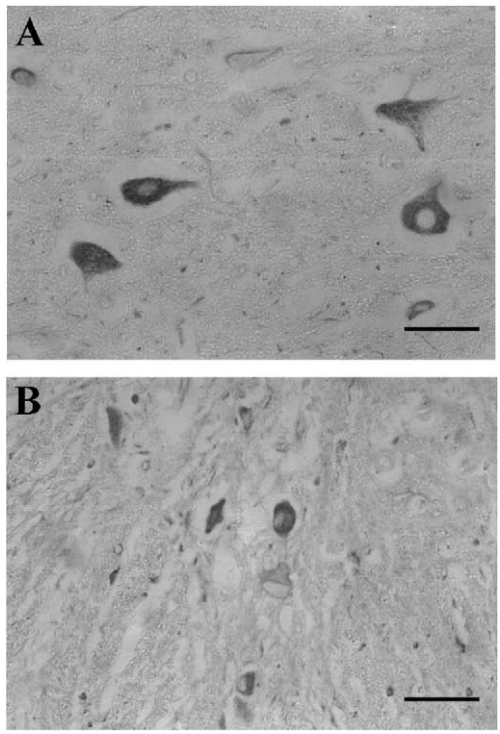

Fig. 3. Motor neuron mGlu1a receptor immunoreactivity in representative autoptic specimens from adult human spinal cord: (A) control case and (B) ALS subject. Scale bars, 100 μm.

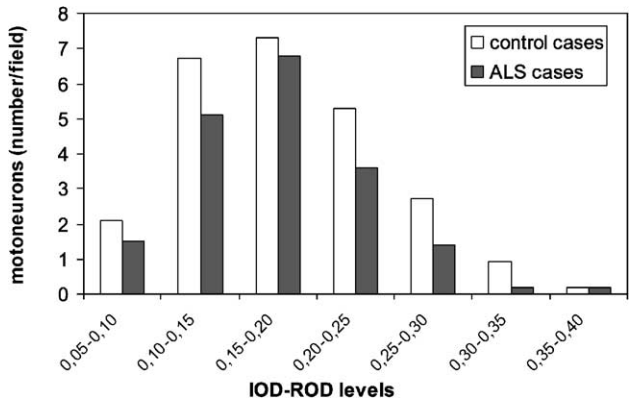

Fig. 4. Semiquantitative analysis of mGlu1a receptor immunoreactivity in spinal cord motor neurons from control (white) and ALS (grey) subjects. IOD–ROD levels represent ranges of integrated optical density values. Total number of motor neurons per field was 25.2±4.9 in control cases and 18.9±3.7 in ALS cases.

usual; moreover, motor neuron number undergo a decrease. However, the immunohistochemical analysis still revealed the presence of mGlu1 receptor motor neuron labelling (Fig. 3B). A moderate mGlu1a immunostaining of cells with apparent astroglial morphology was also observed in some ALS specimens (data not shown).

We conducted a semiquantitative analysis of mGlu1a receptor immunoreactivity in spinal cord motor neurons to verify some possible differences between control and ALS subjects. The number of motor neurons in ALS sections was consistently reduced when compared to control sections. Interestingly, we established that surviving motor neurons from ALS cases express mGlu1a receptor protein at levels comparable to that observed in control cases (Fig. 4).

4. Discussion

By using a reverse transcriptase–polymerase chain reaction technique, we previously found high levels of mRNAs encoding Group I mGlu receptors to be expressed in adult rat spinal cord. In particular, we demonstrated the selective expression of mGlu1a receptor, while mGlu1b receptor subunit was not detectable (Valerio et al., 1997a). In addition, a recent in situ hybridization study characterized the postnatal developmental changes in rat lumbar spinal cord mRNA expression of various mGlu receptor subtypes (Berthele et al., 1999). The mGlu1a and mGlu1d receptor mRNA were found to be colocalized in the spinal cord, with the absence of mGlu1b receptor mRNA confirmed. Use of a pan-mGlu1 probe showed a reduction in mRNA expression in the dorsal laminae between postnatal days 7 and 21, while the motor neuronal signal had slightly increased by postnatal day 21 (Berthele et al., 1999). The present study expands out understanding by further defining the distribution of mGlu1a receptor in the rat and human spinal cords, and determining developmental changes.

Both in rat and in human specimens, mGlu1a immunoreactivity showed a general increase from early gestation to postnatal and adult life. In particular, our results show a weak but detectable immunoreactivity in the ventral horn of rat E15 spinal cord, while sparse labeling was first detected in human foetal spinal cord during the 6th–10th week of gestation. We also observed an increase of mGlu1a receptor expression in the ventral horn of spinal cord with age that culminated in a very strong staining in adult motor neurons of both species. The increasing expression of mGlu1a receptor in motor neurons coincides with the differentiation of the cells in the columns of grey matter to form the groups characteristic of the adult spinal cord, suggesting a possible role for mGlu1a receptor in the maturation process of motor neurons in both rat and human.

Although the precise molecular pathways that cause the death of motor neurons in ALS remain unknown, possible mechanisms include glutamate-mediated excitotoxicity, toxic effects of mutant SOD1, disorganization of intermediate filaments and other abnormalities of intracellular Ca^{2+} regulation in a process that may involve mitochondrial dysfunctions and apoptosis (Julien, 2001). The mechanism of excitotoxic injury of motor neurons appears to involve excessive entry of extracellular Ca^{2+} through the inappropriate activation of glutamate receptors. In particular, a lower expression of GluR2 in motor neurons than in other neurons or an impairment in the editing of the GluR2 mRNA in patients with ALS (Takuma et al., 1999) could lead to the expression of calcium-permeable AMPA receptors, thus altering the ion channels permeability from sodium to calcium-influx and increasing the vulnerability of motor neurons to excitotoxicity (Terro et al., 1998). Increases in cytoplasmic Ca^{2+} may also arise via the activation of Group I mGlu receptors, by release of intracellular Ca^{2+} stores through stimulation of the Gq/phospholipase C pathway. The possible contribution of Group I mGlu receptor to ALS pathogenesis was only indirectly examined until now. In fact, studies conducted in rats (Anneser et al., 1999; Laslo et al., 2001) or in control human subjects (Tomiyama et al., 2001) aimed at measuring Group I mGlu receptor expression in groups of motor neurons resistant or vulnerable during the course of ALS pathology gave controversial results. In situ hibridization data revealed that rat mGlu5 receptors are highly expressed in sacral and thoracic autonomic motor neurons, which are resistant to degeneration in ALS, while they are absent in more vulnerable somatic motor neurons (Anneser et al., 1999). In contrast, the expression of mGlu5 mRNA was found in either vulnerable or spared motor nuclei by Laslo et al. (2001), but with no detectable mGlu5 immunabelling of motor neuron cell bodies (Laslo et al., 2001) as already described in previous reports (Valerio et al., 1997b; Vidnyanszky et al., 1994). A more general consensus arise about the expression of mGlu1 receptors in both rat (Anneser et al., 1999; Berthele et al., 1999; Laslo et al., 2001) and human somatic motor neurons (Aronica et al., 2001; Tomiyama

et al., 2001). Although not quantitatively compared, the mGlu1 receptor appeared expressed in control subjects as well as in ALS cases (Aronica et al., 2001).

Thus, we addressed the second part of our study to investigate the possible functional role of Group I mGlu receptors in the pathophysiology of the excitotoxic damage of motor neurons. AMPA/kainate-mediated toxicity on motor neurons was induced in lumbar spinal cord slices from adult rats cord to assess the possible protective role of a selective Group I mGlu receptor agonist against motor neurons death. We found that 100 μM 3-HPG protected against excitotoxic damage that follows the exposure of spinal cord slices to toxic kainate concentrations. Our data strongly support the major role of mGlu1 receptor in 3-HPG neuroprotection. However, we cannot exclude that also mGlu5 receptors, although poorly expressed in motor neurons or in glial cells, can partially support motor neuron viability. The participation of mGlu5 subtype to motor neuron protection could be even more relevant in ALS spinal cord (Aronica et al., 2001).

While the neuroprotective role of Groups II and III mGluRs is widely demonstrated (Maiese et al., 1995; Buisson et al., 1996; Nicoletti et al., 1996; Kingston et al., 1999), the effects of Group I mGluRs on neurodegeneration is variable depending on the experimental model and the brain areas investigated. It has been suggested that a functional switch from facilitation to inhibition of glutamate release may explain the opposing results obtained with Group I mGluR agonists (Herrero et al., 1998; Nicoletti et al., 1999). It has also been speculated that the neuroprotection elicited by Group I agonists closely depends on the functional status of Group I mGlu receptors: unphosphorylated receptors coupled to PI hydrolysis facilitating glutamate release versus phosphorylated receptors coupled to inhibition of Ca^{2+} channel activity and decreasing glutamate release. This switch from facilitation to inhibition of glutamate release has been observed in several brain areas and involves receptor phosphorylation by PKC (Nicoletti et al., 1999). Indeed, previously studies from our group has demonstrated a role for PKC in the neuroprotection (Pizzi et al., 1996). PKC activation decreases the glutamate-evoked calcium entry into the cells, enhances glutamate transporter activity (Davis et al., 1998) and protects from oxidative stress by elevating the intracellular level of glutathione (Sagara and Schubert, 1998), or by increasing catalytic activity of anti-oxidative enzymes (Doré et al., 1999). All in all, the mechanism contributing to Group I agonist-mediated motor neuron protection has to be elucidated.

Our semiquantitative image analysis data show that the intensity of mGlu1a immunoreactivity in the surviving motor neurons from ALS spinal cord was comparable to that of motor neurons from control subjects. These results are in accordance with the observations of a most recent report by Aronica et al. (2001), which particularly focused on the expression of glial mGlu receptors in control versus ALS spinal cord. While few astrocytic processes showed a weak to moderate mGlu1a receptor immunoreactivity in control spinal cord, the intensity of mGlu1a immunostaining appeared increased in ALS reactive astroglial cells (Aronica et al., 2001). Although the analysis of glial mGlu receptors was not the primary goal of the present study, we also observed that mGlu1a immunoreactivity was clearly detectable in cells with astroglial morphology in ALS spinal cord (data not shown). The physiological role of mGlu1a receptors in glia and their possible contribution to neurodegeneration or protection has still to be investigated.

As it is clear that aetiology of ALS is multifactorial, a combination of treatments attacking different pathogenic pathway may be necessary. The present results demonstrating the neuroprotective properties of Group I mGlu receptor agonists may have a great relevance for possible future therapeutic application to ALS. In fact, motor neurons of ALS patients, even in the last phase of the disease, preserve the capability to express mGlu1a receptors that may be neuroprotective. These observations imply that the remaining motor neurons in ALS patients, although severely damaged, may still positively respond to the administration of drugs acting at mGlu1a receptors. We suggest that these receptors may act as promising targets for the development of effective therapeutic approaches that will slow down the devastating course of the disease.

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