

Amyotrophic Lateral Sclerosis (ALS)

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1. INTRODUCTION

This chapter was originally intended to cover both amyotrophic lateral sclerosis (ALS) and multiple sclerosis, but we will concentrate on ALS. There are nonetheless several reports from the literature that ionotropic glutamate receptor antagonists have beneficial effects in animal models of multiple sclerosis (*1,7,14,58,71,109*) and this could develop into a very promising therapeutic area in the future. The interested reader is referred to the following review (*59*). However, the evidence for involvement of disturbances in the glutamatergic system in ALS is already convincing and has led to the use of the glutamate release inhibitor riluzole in the treatment of this disease. As such, we felt that a book on the therapeutic potential of ionotropic glutamate receptor antagonists and modulators would be incomplete without a chapter on this disease.

2. RATIONALE

ALS, commonly called Lou Gehrig's disease, is a devastating neurological disorder characterized by selective upper and lower somatic, but not autonomic, motor neurone degeneration leading to paralysis and eventually death. The diagnosis of ALS requires the presence of both upper and lower motor neurone degeneration and progressive motor dysfunction. ALS occurs in 1 to 2.5 cases per 100,000 population, affects more men than women, and is most commonly diagnosed in middle age. Other functions such as intellectual abilities and sensory perception are preserved. At advanced stages patients become completely disabled, often requiring ventilatory support and feeding by gas-

trostomy. Death usually occurs within five years of diagnosis and is attributed to respiratory failure or cachexia.

In familial ALS, 20% of the cases are associated with mutations of the copper/zinc superoxide dismutase-1 (SOD-1) gene (73, 75, 83, 87). However, the aetiology of sporadic ALS (90% of patients) remains elusive in spite of intense research. Several theories regarding the pathogenesis of ALS have emerged including glutamate excitotoxicity, free radical oxidative stress, neurofilament accumulation, and autoimmunity.

The role of the glutamatergic system in the pathology of ALS is well documented but existing literature is partially contradictory. This stems both from post mortem, brain imaging and plasma/CSF studies in ALS patients and observations in animal models.

2.1. Pathology

Over a decade ago, it was shown that the pattern of neuronal loss in the spinal cord in patients suffering from ALS resembles that obtained after excitotoxic lesions induced by kainate in animals (43). Injection of kainate to the spinal cord produces damage to motor neurones while NMDA lesions affect mainly dorsal horn neurones (44). Similarly, short exposure to kainate *in vitro* results in selective Ca²⁺-dependent death of motor neurones expressing Ca²⁺-permeant AMPA receptors, whilst dorsal horn neurones are unaffected (102). α -motor neurones in organotypic cultures of rat spinal cord are considerably more sensitive to kainate and quisqualate than to NMDA toxicity (81). This selective motor neurone death is insensitive to inhibitors of voltage-activated sodium channels (VASCs) and voltage-activated calcium channels (VACCs) but is completely blocked by the AMPA receptor antagonists LY300164 and Joro spider toxin (selective for Ca²⁺ permeant receptors - see below) (102). This suggests non-NMDA (AMPA or kainate) receptor involvement.

CSF from ALS patients seems to contain factors with agonistic properties at AMPA receptors (23). Similarly, the number of Fos-like immunoreactivity positive neurones in organotypic cultures of rat lumbar spinal cord was significantly increased in the dorsal horn by addition of CSF from ALS patients as well as by glutamate (100 μ M). These effects were, in both cases, blocked by (+)MK-801 and not by CNQX (55), indicating a predominant role of NMDA receptors.

A possible link between excitotoxicity and autoimmune deficiency in ALS

has been provided by findings that immunoglobulins from ALS patients injected i.p. to rats 24 hrs later produces an increase in glutamate content in the CSF (50,84).

2.2. Serum / CSF Amino Acids

As in other proposed chronic excitotoxic diseases, studies on changes in amino acid levels in ALS are strongly divergent, and there are often contradictions between brain imaging, CSF and plasma / serum levels, even within the same study (65,72). If, as reviewed below, glutamate transporters are involved in the pathogenesis, then it should be stressed that local synaptic concentrations of excitatory amino acids can be expected to be very different from those assessed with such techniques.

In ALS patients with a mild course of the disease, serum glutamate and aspartate content was either normal or slightly decreased, whereas in severely progressing cases serum glutamate and aspartate levels were increased, both effects being accompanied by the opposite changes in glycine concentration (65). Analysis of CSF concentrations in 377 ALS patients revealed the existence of two groups, one with normal glutamate concentrations and one with high glutamate concentrations (41% of ALS patients) which correlated with a spinal onset of the disease, more impaired limb function, and a higher rate of muscle deterioration (90). Similar differences were not seen in 88 other neurological patients and 18 normal controls. Another study also showed correlation between CSF glutamate content and functional impairment assessed with transcranial magnetic resonance stimulation in ALS patients (2).

Proton magnetic resonance spectroscopic imaging of the medulla of ALS patients revealed increased glutamate and glutamine and reduced N-acetyl-aspartate (NAA) and N-acetyl-aspartyl-glutamate (NAAG) content (69). Bulbar symptoms correlated with the changes in glutamate and glutamine but not NAA and NAAG, consistent with the hypothesis of glutamate excitotoxicity in the pathology of ALS (69). In contrast, others have reported that motor cortical and brainstem reductions in NAA content assessed by MRS correlated with the severity of upper motor neurone abnormalities (17).

In the precentral gyrus of ALS patients, glutamate and NAA concentrations were actually decreased, whereas choline and myoinositol concentrations were increased as assessed with localized proton MR spectroscopy (15). Decreased contents of glutamate and aspartate as well as collagen-associated amino acids

such as hydroxyproline, proline, glycine, and hydroxylysine were also seen in the lateral corticospinal tract and the anterior horn of ALS patients (67). Significant reductions in glutamate and aspartate content and increases in reactive astrocytes (i.e., staining with glial fibrillary acidic protein, GFAP) have also been observed in the ALS spinal cord obtained at autopsy (64). These biochemical results corresponded well to pathomorphological neuronal degenerative loss and reactive proliferation of astroglial components in the ALS spinal cord tissue. However, these authors also stressed that their results may only reflect the final pathological and biochemical outcomes of ALS, and give little insight into factors underlying progression of the disease (64).

Another study, with proton magnetic resonance spectroscopy in patients with ALS, in contrast, revealed no differences in glutamate/ phosphocreatine or glutamine/phosphocreatine ratios but decreased NAA which was proposed to reflect neuronal loss or dysfunction (13).

Elevation of taurine has been found in the CNS of ALS patients (54). Taurine is the final product of the metabolic pathway of sulphur amino acids (36) and suggests that intermediate excitatory SAAs could also be increased. Homocysteic acid (HCA) and homocysteine sulphinic acid (HCSA) cause “apoptotic-like” cell death in rat cortical cell cultures (100). This may be related to the fact that cysteine sulphinic acid (CSA) and cysteic acid (CA) potentially inhibit glutamate uptake in synaptosomal fractions from rat cortex (49). Inhibition of high-affinity glutamate transporters by excitatory SAAs, especially CSA and CA, may therefore also be involved in the pathogenesis of ALS.

2.3. EAAT-2

The most widely accepted deficit in sporadic ALS patients seems to be a loss of glial glutamate uptake (excitatory amino acid transporter 2, EAAT-2, GLT-1) evidenced by alteration in protein levels but not changes in mRNA in the spinal cord (37,77,78,82). Results in the motor cortex are less consistent, indicating that glutamate transporter pathology in this disease may also be a more complex phenomenon than previously recognized (77,78,82; c.f. 37). A 43% reduction of high-affinity glutamate uptake rate was also observed in platelets from patients with ALS, compared with normal controls and with patients suffering from different chronic neurological diseases, suggesting a systemic impairment of glutamate uptake in ALS (34).

Two studies showed that mutations in the EAAT2 gene in sporadic ALS are

infrequent and cannot explain the presence of variant mRNA transcripts of EAAT2 in more than 50% of sporadic ALS cases (4,45). Thus, mechanisms other than mutations within the coding region of EAAT2 must be responsible for the low levels of EAAT2 in sporadic ALS. Another study suggested that the loss of EAAT2 is due to multiple aberrant mRNA restricted to neuropathologically affected areas (53). These aberrant mRNAs were proposed to result from processing errors including intron-retention (EAAT2/Int) and exon-skipping (EAAT2/C1) which may then undergo rapid degradation and/or produce a dominant negative effect on normal EAAT2, resulting in loss of protein and activity. Aberrant EAAT2 mRNA species were also detectable in the CSF of ALS patients, early in the disease, and were therefore proposed to be of diagnostic utility (53).

However, another study was unable to confirm a significant correlation between the presence of (EAAT2/C1; EAAT2/Int) and three novel aberrant mRNAs (EAAT2/C2-4) in the motor cortex and ALS pathology (61). Other studies confirmed the presence of similar splice variants in different brain regions of ALS patients, but they were also present in the brains of patients with Alzheimer's disease, Lewy body dementia, and even in normal controls, albeit at lower levels (35,41).

As such, the cause of the decreased expression of EAAT2 in the spinal cord of ALS patients is still a matter of heavy debate. Whatever the mechanism, changes in EAAT2 expression probably results in an increase in synaptic glutamate concentrations leading to excitotoxicity (76,94). This is clearly illustrated by the fact that administration of glutamate transporter antisense in rats leads to a motor syndrome which includes hindlimb paresis (76). In fact, after glutamate loading (60 mg/kg, p.o.) there is a significantly higher increase in glutamate and aspartate levels in plasma in ALS patients than in matched controls (38).

2.3.1. Oxidative Stress

Oxidative reactions seem to be involved in disease processes in the spinal cord of sporadic ALS patients, while there is less evidence for increased oxidative damage in familial ALS (86). It is known that biological oxidants reduce glutamate transporter function and could thereby contribute to build-up of neurotoxic extracellular glutamate levels (68,97). For example, nitric oxide (NO) inhibits glutamate transport, and levels of its stable metabolites (NO_2^- and

NO_3^-) were found to be higher in the serum but not CSF of male sporadic ALS patients, potentially implicating NO in the pathogenesis of ALS, directly or indirectly, and in a sexually dimorphic manner (95). Mutations in SOD-1 (see later) not only reduce the capacity to detoxify superoxide, but can actually catalyse the formation of more deleterious oxidants, such as peroxynitrite (9) and hydrogen peroxide (112). Specific “redox-sensing” elements, consisting of cysteine residues, have been identified in the structures of at least three transporter subtypes (EAAT-1, EAAT-2 and EAAT-3) (97,98).

2.4. Ca^{2+} Permeable AMPA Receptors

One of the crucial questions is why certain motor neurone populations are particularly prone to death in ALS. Although there is no clear cut answer to this question, one of the possibilities is weak Ca^{2+} buffering capacity connected with lowered levels of cytoplasmic proteins responsible for such buffering, such as parvalbumin and calbindin (5,48,79,83,84).

Another explanation is that spinal human motor neurones show low expression of the GluR2 subunit, making AMPA receptors permeable to Ca^{2+} which renders them more vulnerable to excitotoxicity (83,85,93,113). Also important is the fact that editing of mRNA for the GluR2 subunit of AMPA receptors is reduced in the ventral grey matter of patients with ALS, since receptors expressing unedited GluR2 also show very high Ca^{2+} permeability (93). These receptors are also permeant for Zn^{2+} which may play an additional intracellular role in triggering pathological changes (56,111,114).

Other studies in the rat have been unable to reproduce these findings and have therefore attributed increased vulnerability to the greater AMPA receptor density in motor neurones, but it should be noted that they studied cultured cells obtained from early embryonic tissue (103,104). It should however, also be stressed that other neurones expressing low levels of edited GluR2 such as forebrain GABAergic interneurons are less susceptible to glutamate toxicity, indicating that both differences in Ca^{2+} buffering capacity and Ca^{2+} permeability may be important in determining the increased sensitivity of motor neurones (21). Although transgenic mice with deficient Q/R-site editing of the of GluR2 subunit showed neurological deficits, no clear signs of Ca^{2+} -dependent motor neuronal death were observed (33). Similarly, a recent in situ hybridization, RT-PCR and immunohistochemical study in normal adult rats, showed no significant difference in GluR2 mRNA or protein

expression between vulnerable (XII; hypoglossal nucleus) and resistant (III; oculomotor nucleus) cranial motor nuclei (52). However, the presence of mRNA alone is not sufficient to implicate incorporation into functional proteins. Moreover, a recent immunohistochemical double-staining experiment demonstrated that motor neurones expressing edited GluR2, also expressed Ca²⁺-permeable AMPA receptors, indicating that GluR2 is excluded from a subset of AMPA receptors in these cells (102).

2.5. NMDA Receptors

Most of the evidence presented above points to a primary involvement of AMPA receptors in neurodegeneration in ALS. In fact, data on the NMDA receptor role are scarce, and clinical evidence (see below) is rather discouraging. There is however, a decrease in the NR1 subunit in ventral (but not dorsal) spinal cord of ALS patients and a decrease in glycine transporter (106). NMDA receptor expression assessed with [³H]MK-801 binding in spinal cord sections from patients who died of ALS was reduced, and this effect was reversed by phorbol esters. Recent results indicate that abnormal levels or activity of protein phosphatases, including calcineurin, may be involved in this effect in ALS and may play some role in the pathogenesis of the disease (107). The decrease in the glycine transporter could lead to enhanced glycine levels and excessive positive modulation of NMDA receptors. In the spinal cord a decrease of NR2A subunit expression (55-78%) has also been observed (80). The question is whether these deficits are the cause of the neurodegeneration or rather should be interpreted as a secondary consequence of a loss of neurones originally expressing these receptors.

There is evidence that abnormal phosphorylation of protein kinases may be involved in the pathogenesis of ALS and could, for example, cause changes in AMPA and NMDA receptor function. It is again unclear whether this is a primary cause, a secondary event, or a compensatory mechanism – for review see (108).

The neuronal accumulation of phosphorylated neurofilaments (NFs) in ALS patients suggests an alteration of phosphorylation of NFs is also important (30, 73, 105). *In vitro* studies in cultured rat cerebellar granule cells suggest that exposure to a low concentration of glutamate enhances the phosphorylation of NFs, mainly via the AMPA receptor (6). It seems likely that these changes are a consequence rather than the cause of the pathological

changes, but can serve as a relatively selective marker for such changes (63).

2.6. Summary

Although the evidence presented above is partly contradictory, it nonetheless makes the excitotoxic hypothesis of motor neurone cell death in ALS quite attractive. It seems clear that both a diminished function of glutamate transporters such as EAAT2 and an increased susceptibility of motor neurones to glutamate due to increased expression of Ca^{2+} permeant AMPA receptors are important in the aetiology of both familial and sporadic ALS. Other potential targets for therapeutic intervention such as intracellular Ca^{2+} binding proteins, metabotropic glutamate receptors, growth factors and free radicals are out of the scope of the present review.

2.7. Riluzole is a Glutamate Release Inhibitor

In spite of extensive studies, the precise mechanism of action of riluzole (RP 54274) remains elusive. Riluzole, clearly decreases the synaptic release of glutamate and other neurotransmitters (19,22, 57,101) and this effect is probably secondary to inhibition of voltage-activated Na^{+} channels (VASCs) (27,115). Here we discuss the mechanism of action of riluzole *in vitro* as supportive evidence for the role of glutamate in ALS. Later we shall discuss the effects of this compound in animal models of this disease.

Although at first sight the concentrations of riluzole required to block VASCs may seem to be too high (50-150 μM) it seems that it is far more potent in retaining these channels in their inactivated state (i.e., riluzole shifted the steady-state inactivation curve towards more negative values) and would therefore be predicted to be more effective following repetitive activation (89,116).

Riluzole (100 and 300 μM) has been reported to be ineffective against voltage-activated Ca^{2+} channels (VACCs) (116), while others have shown that it blocks N- and P/Q-type channels but not L-type VACCs in DRG cells, with the rank order of efficacy P/Q- > N- >> L-type channels (42). The absolute potency was similar to that at VASCs, and again, lower concentrations shifted steady-state inactivation curves towards more negative values (42). Moreover, another report on acutely isolated neurones from the adult rat neocortex indicates that riluzole is a potent antagonist of both VASCs and VACCs

(92).

At first sight it also seems that riluzole is inactive at the third major family of voltage-activated channels (i.e., voltage-activated K⁺ channels) (VAKCs) (116). However, although the amplitude of the peak outward current through inwardly rectifying VAKCs was not changed by riluzole, the amplitude of the late component was markedly decreased, albeit with low potency (IC₅₀ = 88 μM). Moreover, a very recent report indicates that riluzole may actually be an activator of TREK-1 and TRAAK, two members of a potentially very important new structural family of mammalian background K⁺ channels with four transmembrane domains and two pore regions (29). Riluzole activation of TRAAK was sustained, whereas its activation of TREK-1 was transient, followed by inhibition (29).

In summary, riluzole clearly interacts with a variety of voltage-activated channels, and considering their molecular diversity, it will probably be quite some time until the whole mechanism of action has been characterized.

Riluzole also inhibits NMDA mediated currents in *Xenopus* oocytes with an IC₅₀ of 18 μM, although binding studies failed to detect a direct interaction with the NMDA receptor complex (27). This antagonism is therefore probably not direct, but rather secondary to intracellular, possibly G-protein-dependent processes.

Another recent finding indicates that riluzole not only decreases glutamate release, but also enhances glutamate uptake. Thus, in rat spinal cord synaptosomes, the rate of glutamate uptake was significantly increased in the presence of 0.1 μM and 1.0 μM riluzole, but not at higher concentrations (8). Kinetic analysis demonstrated that riluzole (0.1 μM) decreased the apparent K_m by 21% and increased the V_{max} by 31%. Glutamate uptake was also significantly increased in spinal cord synaptosomes obtained from rats treated with 8 mg/kg (i.p.) of riluzole and sacrificed 4 hrs later. Again, these effects may have been secondary to activation of G-proteins, because they were blocked by cholera toxin (8).

Whatever the receptors / channels involved, riluzole (1-30 μM) attenuated submaximal neuronal death induced by 24 hr exposure to 30 μM kainate or NMDA, but not that by 100 μM NMDA, in cortical cultures (47). Riluzole also attenuated non-excitotoxic oxidative injury induced by exposure to FeCl₃, in the presence of (+)MK-801 and CNQX. Riluzole reduced Fe³⁺-induced lipid peroxidation and inhibited cytosolic phospholipase A(2), indicating that riluzole has direct antioxidative actions. Exposure to higher con-

centrations of riluzole (100-300 μM) for 24-48-hr paradoxically induced neuronal death in a caspase-sensitive manner (47).

3. STUDIES IN ANIMAL MODELS

3.1. SOD-1 Mutants

Although only about 2% of all ALS cases are linked to human SOD-1 mutations (i.e., 1 in 2.5 million population), transgenic models based on this aberration have recently caused a flurry of research in this area, probably partially because of the dearth of alternative models (63). Moreover, it should be noted that mechanistic consequences of this mutation are still not clear (e.g., several studies indicate that SOD-1 mutations found in ALS do not have a reduced capacity to clear superoxide radical and that transgenic mice have, in fact, a gain in overall activity due to the co-presence of wild type SOD-1) (63). Conversely, some of these mutations might actually increase the formation of other more deleterious oxidants (9,112). Whatever the mechanism, these mice do show signs typical for ALS patients, and excitotoxicity seems to be important in mediating the pathology. Also, therapeutic approaches that seem promising in ALS are also effective (for review, see 63). Moreover, it is assumed that many of the secondary processes involved in execution of motor neuronal damage will be the same in the two forms of ALS, because the clinical symptoms and pathology are essentially indistinguishable.

In these mice, ALS-like abnormalities develop at 3-4 months of age while death occurs at 5-6 months. Some researchers have found a decrease in high affinity Na^+ -dependent glutamate uptake between 120 and 150 days of age (20). Interestingly, a significant decrease in V-max (-40%) in spinal cord synaptosomes was seen at 150 days (i.e., one week before the animal's death at 157 days), and this corresponded to a fall in muscle strength. In SOD-1 (but not wild-type) mice mutants, oxidative reactions triggered by hydrogen peroxide inactivate human glutamate transporter EAAT-2 (GLT-1) (99). This supports the notion that in SOD-1-linked familial ALS, neuronal death occurs via an excitotoxic mechanism. In line with this, in SOD-1(G93A) transgenic mice, NMDA- and L-trans-pyrrolidine-2,4-dicarboxylate (LTPD)-stimulated cortical glutamate release were significantly higher, as evidenced by *in vivo* microdialysis and NMR spectroscopy (3). These results suggest impaired glutamate transport in these transgenic mice. Creatine increased

longevity and motor performance of SOD-1(G93A) mice, and decreased glutamate release, possibly, as suggested by the authors, through improved function of the glutamate transporter.

Others also observed a progressive decrease in immunoreactivity for the glial glutamate transporter (EAAT-2) in the ventral, but not in the dorsal horn of the lumbar spinal cord of transgenic mice carrying mutated human SOD-1(G93A) (10). However, the decreases in EAAT-2 occurred after symptoms had already appeared and was accompanied by increased GFAP levels, which suggests that it was not a primary event leading to motor neurone loss. Similar conclusions were also obtained recently by others (24).

Mice transgenic for the SOD-1(G85R) mutation, showed marked symptoms of ALS, characterized by an extremely rapid clinical progression, without changes in SOD-1 activity. Initial indicators of disease were SOD-1 like astrocytic inclusions/aggregates and ubiquitin. The number of astrocytic inclusions escalated markedly as the disease progressed, and this effect was accompanied by a decrease in the glial glutamate transporter (GLT-1) which might imply a role of excitotoxicity in these models. (18).

Glutamate acting via calcium-permeable AMPA/kainate receptors may be a major factor determining vulnerability of motor neurones obtained from SOD-1 mutants (G41R, G93A, or N139K) since CNQX and Joro spider toxin prevented neuronal death (79). The authors concluded that the neurotoxicity in these mice is dependent upon Ca^{2+} entry triggered by glutamate and that this maybe a major factor in the vulnerability of motor neurones in ALS (79). According to Morrison et al. (62) the distribution of GluR2 immunoreactivity in the spinal cord in SOD-1 transgenic mice is not altered and thus cannot be the cause of the selective vulnerability observed in these mice and possibly in ALS patients.

Riluzole (24 or 44 mg/kg/day in diet) - the only drug registered as a disease modifying agent for ALS - delayed the development of motor impairment and prolonged life span in SOD-1 (G93A) transgenic mice (40). This emphasizes the role of an overactive glutamatergic system in the mechanisms of progressive neurodegeneration at least in SOD-1 mutation related familial cases of ALS.

3.2. Spontaneous Mutations

One spontaneously occurring model used to test for potential therapeutic

effects in ALS is the motor neurone degeneration (MND) mouse. Boyce et al. examined the effect of chronic administration of the NMDA glycine site antagonist L-701,324 on the onset and course of neurological impairment in this model (16). Motor deficits starting at 6 months of age were apparent, as a loss of hindlimb reflex extension, impairment of balance, and later abnormalities in walking pattern. L-701,324 (10 mg/kg p.o. daily) given from 4 to 8 months of age did not affect the onset of symptoms or the rate of deterioration of motor performance. This suggests a lack of NMDA receptor involvement in the development of these abnormalities. In contrast, in the same model chronic treatment with NBQX (8 mg/kg daily i.p.) for 3 weeks (starting at age of 24 weeks) significantly improved the behavioural scores (60). These findings suggest again that antagonism of non-NMDA receptors rather than NMDA receptors may be a valuable treatment of ALS.

Progressive motor neuropathy (PMN) is another model of progressive neuropathy resembling some features of ALS (70). The mutation which is spontaneous, recessive and locates to chromosome 13 leads to early paralysis of hind limbs (2-3 weeks of age) and 2-3 weeks later to loss of distal axons, followed by death resulting from respiratory muscle paralysis at 6-7 weeks. Since the progression of neurodegeneration is clearly faster than in SOD-1 transgenic mice, this model is less time consuming but its relevance to ALS pathology needs to be determined (28). PMN mice treated with riluzole (8 mg/kg/day p.o.) showed delayed onset of paralysis and death (46). This treatment also improved motor performance and electromyographic alterations at early disease stages.

A third spontaneous murine phenotype often used as a model of ALS is the Wobbler mouse. However, this model does not show similar pathological changes in the glutamatergic system to those seen in ALS patients or SOD-1 transgenic mice (96) and has, to our knowledge, not been used to test the effects of riluzole.

4. CLINICAL STUDIES

Hence, the evidence given above is probably sufficient to consider glutamate antagonists as plausible neuroprotective treatment of ALS. Unfortunately, the majority of clinical trials with glutamate antagonists, to date, have not been encouraging. Dextromethorphan (NMDA channel blocker) showed no benefit (12,39). Similar negative results were obtained with the glutamate release

inhibitor (GRI) lamotrigine which in a 1.5 year long double blind study in 67 patients, failed to demonstrate a significant effect on disease progression (32).

In contrast, riluzole (also a GRI) has been shown to have moderate efficacy in ALS, mainly expressed as a prolongation of survival in two studies containing together 1104 patients (11,51), but this has not been confirmed by others (19). This agent (®Rilutek) has been registered as a neuroprotective treatment for ALS in several countries.

In a small group of ALS patients the primary peak of the peristimulus time histograms of voluntarily activated single anterior horn motor units was delayed in onset and prolonged in duration (31). This kind of finding has prompted several small open studies to use electrophysiological surrogate parameters to confirm the therapeutic efficacy of riluzole in ALS patients (25,26,88,110). As an example, in 22 ALS patients deficits in the motor unit number estimate and the size of the motor unit action potential in median innervated thenar muscles were partially normalized following treatment with riluzole (110). Riluzole was also shown to partially restore deficient transcranial magnetic stimulation-induced paired-pulse inhibition (PPI) in the first of 4 consecutive 3-month periods of testing, but left paired-pulse facilitation (PPF) unchanged (91). These findings were taken to substantiate the role of anti glutamatergic effects of riluzole in ALS (91). However, others have found that riluzole does not immediately influence intracortical excitability in ALS, but interpreted this to indicate a delayed onset of riluzole's influence on intracortical excitability (88).

In a pharmacovigilance study, riluzole did induce adverse effects in half the patients, the most frequent of which were gastrointestinal disturbances, hepatotoxicity and asthenia. Nonetheless, riluzole was classified as showing an acceptable safety profile (74). There has also been a case report raising the possibility of a causal relationship between riluzole treatment and granulocytopenia (66).

5. CONCLUSIONS

The evidence for a role of excitotoxic processes in disease progression is quite convincing for both familial and sporadic ALS. Diminished function of glutamate transporters, oxidative stress and increased expression of Ca^{2+} permeant AMPA receptors in motor neurones participate in the pathogenesis of this disorder, whereas NMDA receptors seem not to be involved. The glutamate

release inhibitor riluzole is moderately effective, both in the clinic and in animal models of ALS. This provides further impetus to develop more effective agents targeting the glutamatergic system for the treatment of this rare, but devastating disease.

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