J Neurol (1997) 244 [Suppl 2]: S3–S14

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Glutamate, excitotoxicity and amyotrophic lateral sclerosis

Abstract The "glutamate hypothesis" is one of three major pathophysiological mechanisms of motor neurone injury towards which current research effort into amyotrophic lateral sclerosis (ALS) is directed. There is great structural and functional diversity in the glutamate receptor family which results from combinations of 14 known gene products and their splice variants, with or without additional RNA editing. It is possible that motor neurones express a unique molecular profile of glutamate receptors. Abnormal activation of glutamate receptors is one of five main candidates as a final common pathway to neuronal death. In classical acute excitotoxicity, there is influx of Na^+ and Cl^-, and destabilisation of intracellular Ca^{2+} homeostasis, which activates a cascade of harmful biochemical events. The concept of secondary excitotoxicity, where cellular injury by glutamate is triggered by disturbances in neuronal energy status, may be particularly relevant to a chronic neurodegenerative disease such as ALS. Data are now beginning to emerge on the fine molecular structure of the glutamate receptors present on human motor neurones, which have a distinct profile of AMPA receptors. Two important molecular features of motor neurones have been identified that may contribute to their vulnerability to neurodegeneration. The low expression of calcium binding proteins and the low expression of the $GluR_2$ AMPA receptor subunit by vulnerable motor neurone groups may render them unduly susceptible to calcium-mediated toxic events following glutamate receptor activation. Eight lines of evidence that indicate a disturbance of glutamatergic neurotransmission in ALS patients are reviewed. The links between abnormal activation of glutamate receptors and other potential mechanisms of neuronal injury, including activation of calcium-mediated second messenger systems and free radical mechanisms, are emphasised. Riluzole, which modulates the glutamate neurotransmitter system, has been shown to prolong survival in patients with ALS. Further research may allow the development of subunit-specific therapeutic targeting of glutamate receptors and modulation of "downstream" events within motor neurones, aimed at protecting vulnerable molecular targets in specific populations of ALS patients.

Key words Amyotrophic lateral sclerosis · Riluzole · Glutamate receptors · Excitotoxins

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Introduction

The primary pathogenetic processes underlying amyotrophic lateral sclerosis (ALS) are likely to be multifactorial, and the precise molecular mechanisms underlying selective cell death in the disease are, at present, unknown. Evidence is emerging to indicate that cell death in ALS may reflect a complex interplay between genetic factors, toxic activation of glutamate receptors and oxidative stress, which may result in damage to critical target proteins and organelles [1–6]. In some ALS patients, autoimmune mechanisms may contribute to motor neurone injury [7, 8].

This article will highlight: the present state of knowledge relating to glutamate neurotransmission in the human motor system; the concept of excitotoxicity and how this might apply to a chronic neurodegenerative disease such as ALS; current knowledge about the molecular profile of glutamate receptors on human motor neurones; possible reasons for the selective vulnerability of motor neurones to glutamate; the evidence supporting the "glutamate hypothesis" of motor neurone injury; the links between glutamate receptor activation and free-radical-mediated damage.

Normal glutamate neurotransmission

Research during the past decade has shown that glutamate, and in some cases other excitatory amino acids (EAAs), is a major excitatory neurotransmitter in the mammalian nervous system. Of relevance to motor neurone disease (MND) is the probability that glutamate is an important neurotransmitter in several pathways in the human motor system, including the corticospinal tracts [9], excitatory interneuronal pathways in the spinal cord [10] and cortico-cortical association pathways [11]. Postsynaptic glutamate receptors have traditionally been classified into two major categories: ionotropic receptors, which are ligand-gated ion channels, and metabotropic receptors, which are coupled through G proteins to second messenger systems. The ionotropic receptors have been subdivided into three subtypes according to the pharmacological specificity of their preferred agonists: *N*-methyl-D-aspartate (NMDA) receptors; AMPA (α-amino-3-hydroxy-5-methyl-4 isoxazole propionic acid) receptors; and kainate receptors [12]. Fourteen genes have been identified which encode different ionotropic glutamate receptor subunits (Fig. 1) [13–22]. In vivo, each ionotropic glutamate is thought to be composed of four or five subunits arranged as hetero-oligomers or possibly homo-oligomers. The functional properties, including, for example, the ionic permeability of the ion channel, depend on the subunit combination of receptors expressed [23]. Thus, the existence of multiple subunit genes and variations of subunit assembly to form receptor complexes in vivo contribute to the diversity of receptors. In addition, multiple subunit variants can be generated from a single gene by alternative splicing of adjacent exons of glutamate receptor genes as exemplified by the flip and flop variants of the AMPA receptor subunits [24]. A given glutamate receptor gene product and splice variant may be further modified by post-transcriptional RNA editing. This may have significant functional consequences; for example, the change of a single amino acid in the second transmembrane domain of the glutamate receptor channel can determine whether or not the channel is permeable to Ca^{2+} [25, 26].

Thus, the potential number of different subtypes of glutamate ionotropic receptors that may result from combinations of known gene products and their splice variants, with or without additional RNA editing, is very large and it is quite conceivable that a given population of neurones within the CNS, such as motor neurones, will be

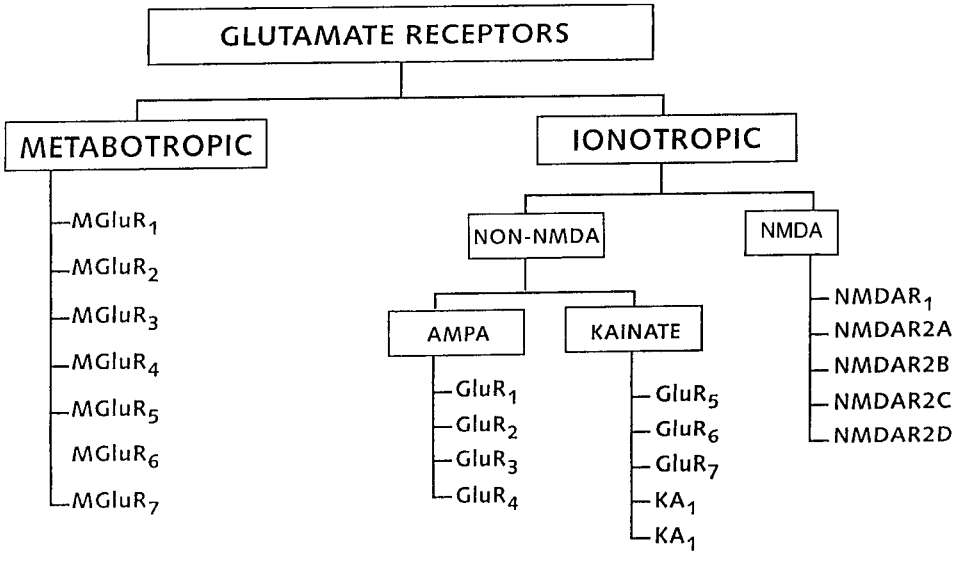

Fig. 1 Classification of glutamate receptors

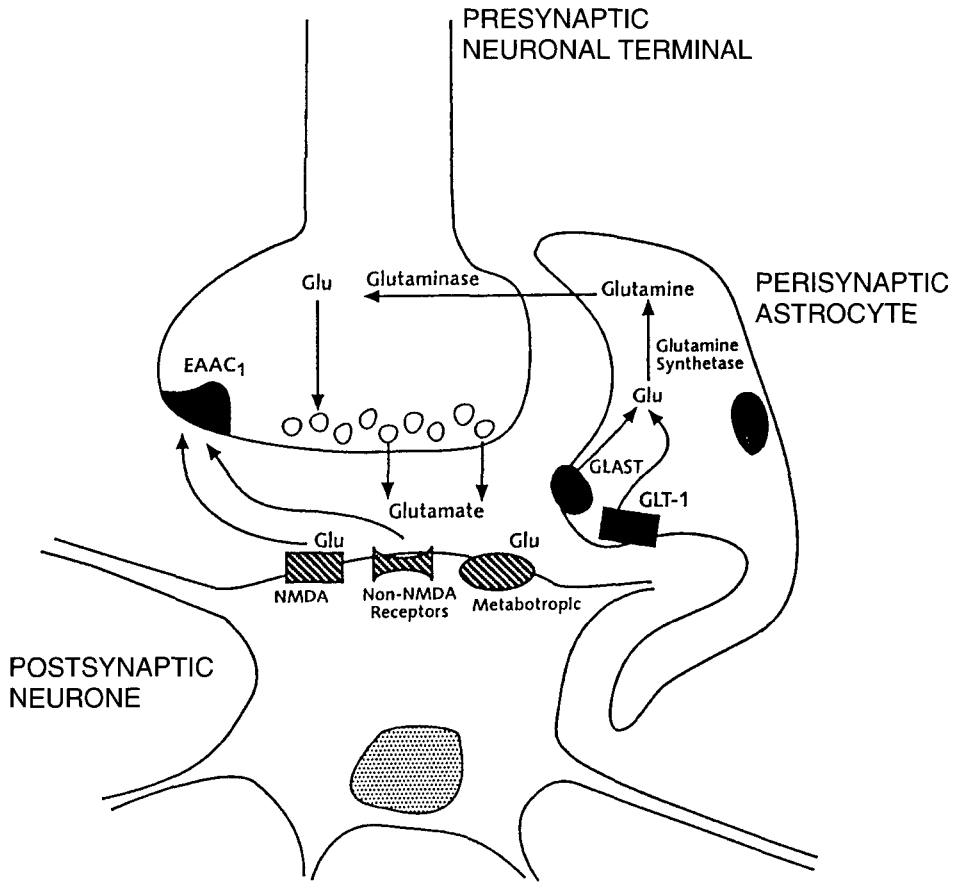

Fig. 2 Normal glutamate neurotransmission

characterised by a relatively unique molecular profile of glutamate receptors.

During normal glutamate neurotransmission (Fig. 2), glutamate is released from the presynaptic neuronal terminal and travels across the synaptic cleft to act on postsynaptic receptors. The excitatory signal is terminated by active removal of glutamate from the synaptic cleft by several types of glutamate re-uptake transporter proteins, which are located on both perisynaptic astroglia and presynaptic terminals. Three rat and four human glutamate transporters have recently been cloned: GLT-1 (human excitatory amino acid transporter $EAAT_2$), which is a widely distributed astroglial-specific glutamate transporter; $EAAC_1$ (human equivalent $EAAT_3$), which has a neuronal localisation; and GLAST (human equivalent $EAAT_1$), which is an astroglial transporter preferentially localised in the cerebellar cortex [27–31]; $EAAT_4$ is a recently cloned human aspartate/glutamate transporter, which is expressed predominantly in the cerebellum [32].

It is thought that the glutamate-glutamine cycle [33] represents an important mechanism for replenishing the neurotransmitter glutamate levels within neuronal terminals. Synaptic glutamate is transported into glia and converted to glutamine by the enzyme glutamine synthetase. Glutamine is then shuttled back to the neuronal terminal where it is reconverted to glutamate by the enzymatic action of glutaminase [33].

Excitotoxicity

Abnormal activation of glutamate receptors is one of five candidates as a final common pathway to neuronal death that are currently of great research interest (Fig. 3). The work of Lucas and Newhouse [34] in 1957 first showed that glutamate can have lethal effects on neurones in the CNS. Subsequently, the term "excitotoxicity" was invoked to describe the neuronal degenerative changes resulting from exposure to glutamate and its EAA analogues [35]. The molecular mechanisms of neuronal injury caused by excessive stimulation of glutamate receptors are beginning to be elucidated and it is clear that glutamate may be toxic to neurones in several ways.

Classical acute excitotoxicity

Studies on neurones in culture have shown that two distinct phases may be involved in excitotoxicity. First, there is acute neuronal swelling caused by depolarisation-medi-

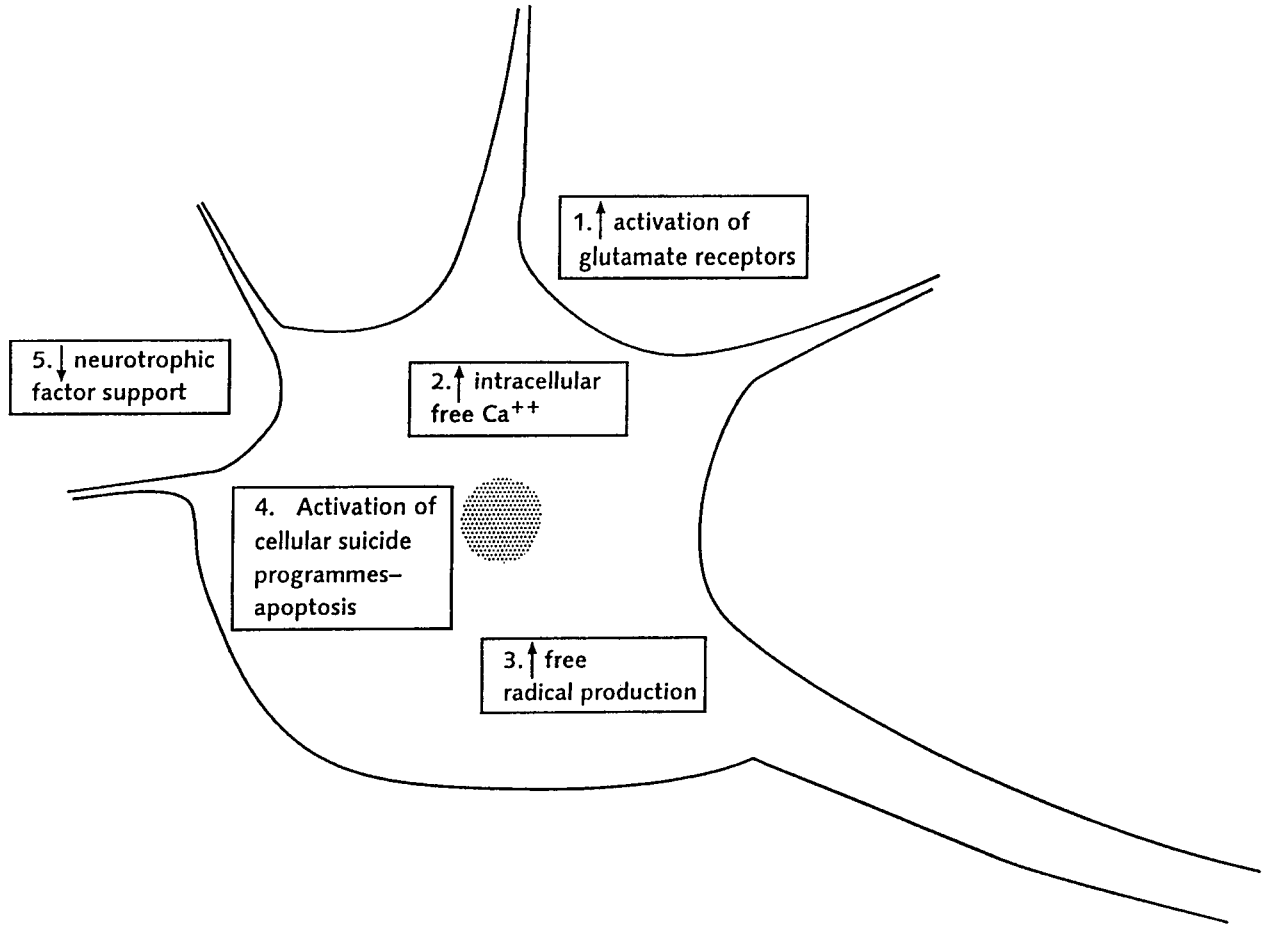

Fig. 3 Final common pathways of neuronal cell death

ated influx of Na^+, Cl^- and water, which appears reversible on removal of the excitotoxin [36]. Second, there is excessive influx of Ca^{2+}, either directly through glutamate receptor ionotropic channels or through voltage-gated calcium channels following depolarisation of the neurone [37]. Normally there is very tight regulation of intra-neuronal free Ca^{2+}, maintaining the level of free Ca^{2+} below 0.1 μM [38]. After excessive stimulation of glutamate receptors, there is destabilisation of intracellular Ca^{2+} homeostasis, which activates a cascade of cytotoxic biochemical events including inappropriate activation of several enzyme systems (lipases, phospholipases, endonucleases, calpains, nitric oxide synthase, protein kinase C and xanthine oxidase). These processes can injure the neurone both directly and through the generation of free radicals [39]. Until recently, the NMDA subtype of glutamate receptor, with its calcium permeable cation channel, was primarily implicated in excitotoxic injury to neurones [40]. However, it is now apparent that activation of non-NMDA (AMPA/kainate receptors) may also result in excitotoxic effects, particularly with more prolonged exposure to receptor agonists [41].

Secondary or "weak" excitotoxicity

Following the original observations of Novelli et al. [42], it is now apparent that excitotoxicity can be viewed not merely as a primary disease mechanism, but as a secondary phenomenon triggered by disturbances in neuronal energy status. A neurone that is compromised by some primary pathological process may show impaired glucose metabolism, reduced ATP production and dysfunction of Na^+/K^+-ATPases, which are necessary to generate a normal resting membrane potential. Thus, for example, the voltage-dependent Mg^{2+} block of the NMDA receptor channel may be eliminated, resulting in over-activation of NMDA receptors by normal endogenous glutamate levels. Recently, Riepe et al. [43] extended the concept of excitotoxicity under conditions of chronic neuronal energy blockade. They studied the effects of exposure to glutamate of hippocampal slice preparations maintained under conditions of chronic inhibition of oxidative phosphorylation. In this system, failure of neuronal ion exchange, particularly Na^+ transport, together with disturbed interaction between neurones and glia, were the main processes that resulted in neuronal death.

The concept of secondary, or weak, excitotoxicity is theoretically appealing in relation to a chronic neurode-

generative disease such as ALS. This mechanism could lead to toxic activation of glutamate receptors without any abnormality in the level of glutamate, or alteration in glutamate receptors. It could account for the relative anatomical specificity of ALS, and a wide variety of primary abnormalities in motor neurone physiology could result in susceptibility to the toxic effects of glutamate. In addition, therapy targeted at glutamate receptors may retard the progression of neuronal injury, whatever the primary disease process [44].

Inhibition of cystine transport

Recently, it has become apparent that glutamate can have an indirect toxic effect by depletion of intracellular glutathione [45]. The cystine carrier transports cystine into cells and also transports glutamate out of the cell, the driving force for this exchange being provided by the glutamate gradient. Cystine and glutamate compete for binding to the carrier and an elevation of extracellular glutamate will result in decreased intracellular cystine transport. Cystine is a vital precursor of intracellular glutathione, which protects the cell from oxidative damage and also has a direct effect on neuronal excitability through an effect on K^+ conductance [46].

Free radical production

Activation of glutamate receptors is one of the main routes to free radical production by calcium-dependent activation of the arachidonic acid cascade, nitric oxide synthase and calpain [47, 48]. Reactive oxygen species can lead to cumulative cellular injury and death, by damage to constituent proteins, lipids and DNA, with impairment of function of essential macromolecules and organelles.

Time course of excitotoxic neuronal death

There is now a substantial body of evidence that glutamate receptor agonists can produce neuronal death following acute exposure. There are also some emerging lines of evidence that excitotoxic mechanisms can potentially produce chronic neurodegenerative pathology. Excitotoxic effects with a prolonged time course have been shown after exposure of organotypic CNS tissue cultures, or striatum or ventricular system of rats, to low concentrations of the EAA agonist quinolinic acid [49, 50]. Chronic pharmacological blockade of the glutamate re-uptake transporter system in spinal cord explants from rats results in degeneration of motor neurones over a time course of weeks [51]. Activation of glutamate receptors under physiological conditions is known to produce long-lasting changes in specific aspects of cellular biochemistry, resulting, for example, in changes in synaptic efficiency known as long-term potentiation [52]. Also, glutamate receptor activation of neurones under pathological, as well as physiological, conditions may alter gene expression in a long-lasting manner [53]. As discussed above, activation of cell-surface glutamate receptors is a particularly important pathway for free radical damage, and oxidative damage to intracellular proteins, lipids and DNA may be cumulative [54].

Glutamate receptor profile of human motor neurones

Motor neurones have a high density of glutamate receptors [55–57] and in culture are susceptible to toxic effects following activation of either NMDA or non-NMDA glutamate receptors [58]. The distribution and density of ionotropic glutamate receptor subtypes and the glutamate re-uptake transporter system in the normal human motor system have been systematically studied using quantitative autoradiography with specific radioligands [55, 59–62]. The key important findings are: (1) glutamate receptors of both NMDA and non-NMDA (AMPA/kainate) subtypes are expressed in the motor cortex, brainstem and spinal cord regions of the normal human motor system; (2) focal areas of high binding ("hot spots") of [^{3}H]MK-801 (NMDA receptors) and [^{3}H]D-aspartate (glutamate transporter system) are co-localised with lower motor neurone somata; (3) motor neurone groups that tend to be spared in ALS [e.g. oculomotor (III) nucleus] express a lower density of NMDA receptor binding sites and a higher density of AMPA binding sites compared with motor neurone groups vulnerable to the disease. These findings indicate differences in normal glutamate neurotransmission in spared and vulnerable motor neurone groups.

Data are now beginning to emerge on the fine molecular structure of glutamate receptors present on human motor neurones. Immunocytochemical studies with subunit specific antibodies have shown that human motor neurones have a relatively distinct profile of AMPA receptor subunits, with low levels of expression of the $GluR_1$ protein, high levels of $GluR_{2/3}$ and moderate levels of $GluR_4$ [56]. In situ hybridisation to study the cellular expression of AMPA subunit mRNAs has shown that human spinal motor neurones express $GluR_1$, $GluR_3$ and $GluR_4$ but have no detectable expression of the mRNA for $GluR_2$ [63]. The $GluR_2$ subunit has a very important role in determining the calcium permeability of AMPA receptors [64]. Most native AMPA receptor subunit hetero-oligomers in the human CNS include the edited form of $GluR_2$, which renders them impermeable to calcium [65]. Only a few groups of cells in the mammalian CNS appear to express calcium-permeable AMPA receptors; these include Bergmann glia in the cerebellum and a subpopulation of hippocampal neurones [64–66]. It has been shown that neuronal subpopulations expressing atypical AMPA receptors

that lack $GluR_2$, which gate calcium permeable ion channels, exhibit heightened vulnerability to non-NMDA agonist toxicity [67]. Thus, the lack of $GluR_2$ expression by human motor neurones and the resulting likely permeability of their AMPA receptors could potentially render this cell group vulnerable to excitotoxic injury by increasing calcium influx during glutamate receptor activation.

Further work needs to be undertaken to elucidate the molecular profile of NMDA and kainate receptors, as well as the glutamate transporter system, in relation to human motor neurones. In addition, other molecular features of glutamate receptors that may be important in contributing to cell-specific excitotoxic vulnerability, which include the expression of flip versus flop variants of AMPA receptors [68] and the expression of particular splice variants of the $NMDAR_1$ subunit [69], have not yet been studied in the human motor system.

Selective vulnerability of motor neurones

To be plausible, the "glutamate hypothesis" of motor neurone injury must explain how motor neurones can be selectively damaged by a disturbance of the glutamate neurotransmitter system, given the fact that EAA receptors are widely distributed throughout the CNS. Motor neurones differ from many other groups of cells in the CNS by their large size, their high ratio of axonal length to cell soma diameter, their high metabolic rate, their high content of neurofilament proteins and free radical scavenging enzymes [70–72]. In relation to glutamate toxicity, two cell-specific molecular features of human motor neurones have been identified that may render this cell group unduly susceptible to calcium-mediated toxic events following glutamate receptor activation. The first feature is the low expression of $GluR_2$ and the resulting likelihood that human motor neurones express atypical, calcium-permeable AMPA receptors, which is discussed in the preceding section. The second feature is that human motor neurones that are vulnerable in ALS do not express the calcium-binding proteins parvalbumin and calbindin D28K [73]. These proteins buffer intracellular Ca^{2+} and may play an important role in the protection of neurones from calcium-mediated injury following activation of glutamate receptors. A direct relationship has been shown between cellular Ca^{2+} buffering capacity and resistance to glutamate neurotoxicity [74]. These two molecular features may, in combination, render human motor neurones particularly susceptible to calcium toxicity following AMPA receptor activation. Thus, a plausible explanation is beginning to emerge whereby disturbances of glutamate neurotransmission in ALS may cause selective injury to motor neurones.

Evidence for dysfunction of the glutamate neurotransmitter system in ALS

The evidence that a disturbance of glutamate transmission may be present in ALS has been discussed in several recent reviews [1–3]. The key points will be highlighted here, with emphasis on recent developments.

Glutamate levels in CNS tissue, cerebrospinal fluid and plasma of ALS patients

Several groups have found significant reductions in the levels of glutamate in several CNS regions of ALS patients [75–77]. Reductions in the levels of aspartate, *N*-acetyl-aspartyl glutamate (NAAG) and *N*-acetyl-aspartate (NAA) in the spinal cord have also been reported [77]. This has led to the hypothesis that there may be an underlying defect in the metabolism, transport or storage of glutamate. Several studies have shown the level of glutamate in the cerebrospinal fluid (CSF) to be increased in ALS patients [78, 79], although not all groups have confirmed this finding [80]. The reason for these discrepancies may relate, in part, to the heterogeneity of the MND patients in some of the studies, and also to technical difficulties in measuring glutamate in biological samples [81]. A recent study has indicated that the elevation of CSF glutamate may only be present in a subset of approximately 30% of patients with ALS, the remainder of patients having levels within the control range [79]. The identification of a subgroup of MND patients with high CSF glutamate levels may be important in evaluating the clinical response to anti-glutamate therapeutic agents. It is of interest that the CSF of ALS patients has been shown to be toxic to neurones in culture, apparently via activation of non-NMDA glutamate receptors [82]. It has not yet been established whether such toxicity correlates with the level of CSF glutamate.

Controversy exists regarding fasting plasma glutamate levels in ALS. Plaitakis and Caroscio [83] reported that fasting plasma glutamate levels were increased by approximately 100% in ALS patients compared with controls, and that oral glutamate loading produced plasma levels which were higher in the ALS group. Iwasaki et al. [84] agreed with these findings, but others have found normal plasma glutamate levels in ALS patients [79, 80].

Abnormalities of glutamate transport

Rothstein et al. [85] showed a specific functional defect in the Na^+-dependent glutamate uptake system of synaptosomes obtained from spinal cord or affected regions of brain in ALS patients. This led to the hypothesis that inefficient synaptic clearance of glutamate could result in excessive activation of EAA receptors with resulting toxic

effects on motor neurones. It has been shown that "knock-out" of the glial glutamate transporters GLT-1 and GLAST using chronic antisense oligonucleotide administration in vivo produces elevation of extracellular glutamate levels, excitotoxic neurodegenerative changes, and progressive paralysis [86]. Studies using antibodies to synthetic peptides from three of the cloned glutamate transporters showed a substantial loss of the astroglial GLT-1 immunoreactive protein in ALS, with a distribution largely confined to the areas of pathology in the motor cortex and spinal cord [87]. There was a 70% decrease in GLT-1 expression in motor cortex from ALS patients, and in about 25% of the ALS cases the loss of expression of the GLT-1 protein was dramatic, with no accompanying depletion of another glial specific protein, glial fibrillary acidic protein (GFAP), or GLT-1 mRNA [88]. The reason for the selective loss of the glial transporter protein in ALS is, at present, unknown, and it is by no means established that the abnormality of glutamate transport is a primary pathogenic factor. It also appears that GLT-1 expression may be subject to rapid alteration post-mortem (Shaw, unpublished observations), so results from human post-mortem studies should be interpreted with caution. However, it remains possible that in a proportion of patients with ALS there is an abnormality in the synthesis or turnover of the protein. Alternatively, GLT-1 could be selectively damaged by other pathophysiological processes, such as oxidative stress, given the known sensitivity of glutamate transport to damage by free radicals [89]. A further possibility is that GLT-1 expression is down-regulated following motor neurone degeneration.

Densities of glutamate receptor binding sites

Autoradiographic studies have shown an increased density of binding sites for NMDA and non-NMDA receptor ligands in ALS, particularly in the intermediate grey matter of the spinal cord and deep layers of the motor cortex [61, 62]. This may reflect increased excitatory drive to surviving motor neurones.

Experimental studies

Various experimental studies have provided evidence that glutamate receptor agonists may contribute to motor neurone injury. For example, intrathecal injection of the EAA agonist kainic acid in mice preferentially injures anterior horn cells and induces within them the formation of abnormally phosphorylated neurofilaments, a cytoskeletal abnormality that has been documented in MND [90]. Using a tissue culture model in which organotypic rat spinal cord is maintained under conditions of chronic glutamate uptake inhibition, motor neurone toxicity is produced with a subacute time course [51]. In this experimental paradigm therapeutic agents have been evaluated for neuroprotective effects on motor neurones. It appears that drugs which inhibit glutamate release, which block glutamate synthesis, or which act as non-NMDA receptor antagonists are the most potent neuroprotective agents, and certain antioxidants or inhibitors of nitric oxide synthesis can also exert a modest neuroprotective effect [91].

Exogenous excitotoxins

Exogenous excitotoxins have been implicated in the aetiology of specific forms of human motor system degeneration, and this has led to the suggestion that similar mechanisms may underlie ALS itself. Human lathyrism is thought to be caused by ingestion of *B-N*-oxalyl-amino L-alanine (BOAA), a glutamate analogue present in the chickling pea (*Lathyrus sativus*) [92]. Victims of this disorder develop upper motor neurone signs predominantly in the lower limbs. There have been few pathological studies, but there is evidence of degeneration of the corticospinal tracts in the spinal cord [93]. Seven per cent of patients have clinical evidence of lower motor neurone dysfunction [94] and inclusion bodies have been described within the anterior horn cells of victims of lathyrism [95]. Until recently, one of the major hypotheses for the high incidence of ALS and parkinsonism dementia in the Western Pacific was that neurotoxicity may have resulted from the use of the seed of the false sago palm (*Cycas circinalis*) for food and medicinal purposes by the native population [96]. One constituent of the cycad seeds is *B-N*-methyl-amino L-alanine (BMAA), which is a potential excitotoxin capable of activating several types of glutamate receptor. However, the balance of recent evidence suggests that BMAA acting as an excitotoxin is not likely to be the cause of the human disease [97], although it is noteworthy that this compound can exert other neurotoxic effects, including disruption of mRNA metabolism [98]. The excitotoxin domoic acid, which is a selective kainate receptor agonist, was responsible for an outbreak of food poisoning following consumption of contaminated mussels in a Canadian population [99]. While the hippocampus and amygdala suffered the most severe neurotoxic damage, some of the affected individuals developed limb weakness, suggestive of either lower motor neurone injury or a motor polyneuropathy [100].

Positron emission tomography studies

Abnormalities in the contralateral cortical increase in cerebral flow caused by freely selected upper limb movements have been shown in ALS patients compared with controls [101]. In the ALS group there was significantly greater activation in several cortical areas, implying inappropriate activation of pyramidal tract neurones, outside

the normal somatotopic representation of the moving upper limb. This suggests an imbalance between excitatory and inhibitory neurotransmission in the cortex in ALS patients.

Neurophysiological studies

Transcranial magnetic stimulation of the motor cortex has shown abnormalities in a proportion of patients with ALS, indicating the presence of hyperexcitability of motor neurones in these patients [102, 103].

Therapeutic modulation of glutamate neurotransmission

Two recent trials using the drug riluzole have shown positive effects in terms of improved survival of patients with ALS [104, 105]. Riluzole interferes with pre- and postsynaptic glutamate neurotransmission via a complex mechanism of action involving the blockade of voltage-sensitive Na^+ channels, ionic flux through NMDA channels, and possibly also interaction with G proteins [106–110]. Riluzole inhibits glutamate release, decreases EAA-evoked firing of rat facial motor neurones, and exerts neuroprotective effects in experimental models of acute and chronic neurodegenerative disease [58, 110–113]. An interesting property of riluzole is that its binding affinity is several hundred-fold higher for the inactivated state of Na^+ channels compared with the activated state [114]. This state-dependent drug affinity means that riluzole can be expected to preferentially block depolarised hyperactive neurones, because their Na^+ channels are more often in the inactivated state compared with Na^+ channels of normal neurones.

The recently published clinical trial of riluzole showed (using a Cox proportional hazards model of analysis) a 35% increase in survival at 18 months in the group receiving the optimal dose of riluzole compared with placebo [105]. This trial did not show a significant effect of riluzole on muscle strength or disability scores, although the earlier trial did report a beneficial effect on decline of muscle strength [104].

Links between glutamate receptor activation and free-radical-mediated damage

Activation of glutamate receptors and subsequent calcium-dependent second messenger systems is an important pathway for free radical production within neurones [5, 6]. Free radicals are one of the main potential causes of age-related deterioration in neuronal damage, and accumulation of oxidative damage may contribute to the delayed onset and progressive nature of neurodegenerative diseases [54]. There is considerable interest in the role of free radicals in motor neurone injury following the discovery that some patients with familial ALS have point mutations in the gene on chromosome 21 that encodes Cu,Zn superoxide dismutase (SOD1) [115]. The normal role of SOD1 is to catalyse the removal of superoxide radicals that can contribute to cellular oxidative damage [116, 117]. The molecular mechanisms of selective motor neurone injury in the presence of SOD1 mutations are not understood, but recent evidence, including studies of transgenic mice expressing mutant human SOD1, suggests that the mutant protein has acquired some "toxic gain-of-function" [118, 119]. One hypothesis for this toxic effect is that the mutant SOD1 protein may alter the sensitivity of motor neurones to glutamate neurotransmission by, for example, inducing mitochondrial dysfunction [4]. Interestingly, lower motor neurones in SOD1 transgenic mice develop pathological changes resembling excitotoxic effects [120], and the glutamate inhibitor riluzole prolongs the survival of affected animals [121].

There is emerging evidence that oxidative stress may contribute to motor neurone injury in the sporadic form of ALS. Thus,

1. Protein carbonyl levels (an index of oxidative damage to protein) are increased in spinal cord [122] and frontal cortex [123] of ALS patients compared with control cases.
2. The activity of the free-radical scavenging enzyme glutathione peroxidase [124], the protein expression of SOD1, Mn SOD and catalase [72] are all increased in ALS spinal cord, as is the expression of SOD1 mRNA in individual spinal motor neurones [125]. These changes may reflect a compensatory response to oxidative stress.
3. The level of iron is increased in ALS spinal cord, which could potentially contribute to oxidative cellular injury [126, 127].
4. Astrocytes in ALS spinal cord show increased expression of metallothioneins [128].
5. Antioxidant therapy may have a modest beneficial effect on the clinical course of sporadic MND [129].

An important consideration is that some proteins that appear particularly sensitive to free radical damage are very important in the regulation of glutamate neurotransmission; these include glutamine synthetase [130] and the glial glutamate transporter GLT-1 [89], as discussed above. Exposure of astrocyte cultures to low-level oxidative stress results in selective damage to the high-affinity glutamate transporter system without significant cytotoxicity.

Conclusions

Whatever the primary pathophysiological process (or processes) underlying motor neurone injury in ALS, the glutamate neurotransmitter system is likely to remain an important target for therapies aimed at retarding the

pathological progression of the disease. Further work aimed at identifying specific subgroups of ALS patients, and at elucidating a more detailed picture of the precise molecular structure of glutamate receptors located on motor neurones, may allow subunit-specific therapeutic targeting aimed at specific populations of ALS patients. Further exploration of the events within motor neurones occurring "downstream" of glutamate receptor activation may allow the development of synergistic therapeutic strategies aimed at protecting the underlying vulnerable molecular targets.

Acknowledgements P.J.S. is supported by the Wellcome Trust as a Senior Fellow in Clinical Science. P.G.I. is supported by the Medical Research Council.

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