Invited Review Article

Glutamate Receptors in Neuroinflammatory Demyelinating Disease

Christopher Bolton[1] and Carolyn Paul[2]

[1] Centre for Biochemical Pharmacology and Experimental Pathology, John Vane Science Centre,
 St Bartholomew's Hospital Medical School, Charterhouse Square, London EC1M 6BQ, UK
[2] Faculty of Applied Sciences, University of the West of England, Frenchay Campus, Coldharbour Lane,
 Bristol BS16 1QY, UK

Multiple sclerosis (MS) is a chronic demyelinating disease of the human central nervous system (CNS). The condition predominantly affects young adults and is characterised by immunological and inflammatory changes in the periphery and CNS that contribute to neurovascular disruption, haemopoietic cell invasion of target tissues, and demyelination of nerve fibres which culminate in neurological deficits that relapse and remit or are progressive. The main features of MS can be reproduced in the inducible animal counterpart, experimental autoimmune encephalomyelitis (EAE). The search for new MS treatments invariably employs EAE to determine drug activity and provide a rationale for exploring clinical efficacy. The preclinical development of compounds for MS has generally followed a conventional, immunotherapeutic route. However, over the past decade, a group of compounds that suppress EAE but have no apparent immunomodulatory activity have emerged. These drugs interact with the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-isoxazolepropionic acid (AMPA)/kainate family of glutamate receptors reported to control neurovascular permeability, inflammatory mediator synthesis, and resident glial cell functions including CNS myelination. The review considers the importance of the glutamate receptors in EAE and MS pathogenesis. The use of receptor antagonists to control EAE is also discussed together with the possibility of therapeutic application in demyelinating disease.

INTRODUCTION

Dedicated research by numerous scientific groups into the causes and treatment of the human demyelinating disease multiple sclerosis (MS), the most common disabling neurological condition of European, North American, and other temperate climates, has been ongoing for many decades. MS affects relatively young individuals, with a female to male ratio of approximately 2 : 1. The disease is considered to involve central nervous system (CNS) autoantigen-directed T lymphocytes acting in concert with a genetically determined susceptibility and exposure to environmental induction factors [1]. Progress has been made to advance understanding of the disease process and offer effective methods of control. However, there remains a lack of fundamental knowledge on the primary aetiology of MS and a paucity of treatments to alleviate symptoms and ultimately improve quality of life for the patient.

The development and refinement of the inducible animal disease experimental autoimmune encephalomyelitis (EAE)

has provided a reliable model for the study of MS offering pathological and neurological features of striking similarity to the human condition. The principal characteristics in common include immunoregulatory defects, neurological disabilities, blood-brain barrier (BBB) damage with associated vasogenic oedema, inflammatory cell invasion of the CNS parenchyma and, in the chronic models, demyelination and macroscopic plaque formation [2]. However, the premise that EAE is strictly a model for MS must remain, not least because of the obvious species differences and timescale of disease appearance and progression, but also because of factors such as the divergence in identity of the causative agents and the unpredictable patterns of clinical deficits experienced by patients.

Animal counterparts of human disease, whether spontaneous or inducible, have inherent limitations and EAE is no exception. However, the model does provide an extensively validated and useful in vivo system of immune cell-mediated demyelination complete with quantifiable neurological deficits. In particular, the model provides the

opportunity to evaluate potential new therapies for MS treatment and explore novel approaches to drug design, identify new targets, and add to the growing number of drugs in clinical trials.

The search for compounds with the ability to modify the onset and development of EAE have invariably focused on immunomodulatory agents [3]. However, over the last few years, a group of established compounds have emerged, with the ability to dramatically improve the course of EAE but without apparent immunosuppressive activity. The compounds interact with members of the neuronal ionotropic glutamate receptor family comprising the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-isoxazolepropionic acid (AMPA), and kainate receptors (Figures 1(a)–1(c)). The 3 types of receptors are ligand-gated ion channels, named according to their specific agonists, which control the most rapid synaptic events in the nervous system through receptor-channel complex-mediated events.

Our original studies of 1994 [4, 5] were the first to implicate the NMDA receptor in the pathogenesis of EAE. Over the intervening years, there has been compelling evidence, reviewed below, to confirm an important role for the NMDA receptor in the disease. Additional investigations have strongly indicated that AMPA receptors play a part in the development of EAE and, of particular interest, more recent unpublished studies have shown altered receptor expression in CNS tissues from MS patients (T. Smith, personal communication). The amino acid glutamate is the main agonist of the receptors and has been implicated in the pathogenesis of neuroinflammatory disease [6, 7]. Hence, the discovery of NMDA/AMPA receptor involvement in both EAE and MS offers a plausible association between the receptors, the amino acid, and development of both diseases.

GLUTAMATE IN EXPERIMENTAL AND HUMAN NEUROINFLAMMATORY DISEASE

Olney, in the late 1960s [8], was the first to recognise that the ubiquitous neurotransmitter, glutamate, when present in excess, has the potential to be excitotoxic. Glutamate formation is regulated by the enzyme glutamate dehydrogenase which catalyses the reaction of α-oxoglutarate with ammonia [9]. The agonist concentration can be abnormally increased by accelerating the reversible formative reaction that is controlled by pyridine nucleotide coenzyme activity. Glutamine synthetase controls the incorporation of ammonia into glutamate to form glutamine and the activity of the enzyme can be dramatically increased or decreased in the presence of excess divalent cations, including magnesium (Mg^{2+}). Glutamate is stored in synaptic vesicles and released by calcium (Ca^{2+})-dependent exocytosis. Sodium-dependent, plasma membrane transporter proteins EAAC1 (EAAT3) and EAAT4, present mainly in neurons, and GLT-1 (GAAT2) and GLAST (GAAT1), expressed predominantly in glial cells, facilitate cellular uptake of glutamate and accumulation in synaptic vesicles [10].

Several studies have demonstrated glutamate involvement in the pathology of EAE, and also MS, offering the

clear potential for aberrant ionotropic receptor activation. In particular, the glutamate antagonist amantidine has been shown to reduce the relapse rate in individuals with MS [11]. Also, Stover et al [12] have reported elevated glutamate levels in the cerebrospinal fluid from MS patients. Interestingly, the elevation was similar to concentrations recorded in myelopathy and, perhaps more surprisingly, greater than noted during cerebral ischaemia. However, and in contrast to the previous findings, Klivenyi et al [13] found no differences between cerebrospinal fluid glutamate concentrations in MS and control samples despite elevated levels in both groups.

Enhanced concentrations of the agonist may result from malfunctioning of activated astrocytes normally efficient at controlling excess glutamate through regulation of the metabolising enzymes glutamate dehydrogenase and glutamine synthetase, which become down-regulated during inflammatory conditions such as EAE [14, 15]. The amount of CNS glutamate may also be increased in EAE by abnormal changes in neuronal and glial glutamate transporter levels [16] which, under pathological conditions, are either inoperative or acting reversibly to raise extracellular concentrations of the agonist. Glutamate leakage from the serum across the compromised BBB during EAE plus infiltrating inflammatory leukocytes and activated resident microglia with the potential to synthesise and release glutamate would provide a continuous, local supply of the agonist. Also, microglia are known to generate reactive oxygen and nitrogen species that impair glutamate uptake mechanisms. The constant availability of glutamate would induce upregulation of its receptors and, ultimately, the synthesis of mediators responsible for neuronal dysfunction [12, 16–19]. Indeed, a recent study in EAE found that prophylactic administration of riluzole, an inhibitor of glutamate-dependent neurotransmission, reduced neurological severity, inflammation, demyelination, and axonal damage strongly suggesting a broad role for the enhanced presence of glutamate in the pathology of the disease [20].

In a novel approach to account for the increase in CNS glutamate concentrations during neuroinflammation, Rose et al [21] have suggested a mechanism that would operate through the actions of two enzymes, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), both of which have been located in MS lesions. COX-2-derived prostanoids, which exist at high concentrations in EAE and MS CNS tissues [22–24], stimulate glutamate release from CNS-derived cells [25, 26]. Additionally, nitric oxide (NO), from iNOS, can increase COX-2 [27], plus reactive oxygen species (ROS) [28], to react with NO to produce peroxynitrite ($ONOO^-$) [29] that inactivates the glutamate transporters [30, 31]. In addition, $ONOO^-$ directly damages myelin, oligodendrocytes, and axons [32], and therefore plays a predominant role in the pathogenesis of EAE [33].

The evidence is unequivocal as to the consequences of excitotoxic glutamate levels in the CNS of patients with neuroinflammatory-based disease and that target tissues require protection from the sustained biochemically-mediated attack. Interestingly, in EAE, work by Schori et al [34] supports a T-cell-dependent, self-protective immune
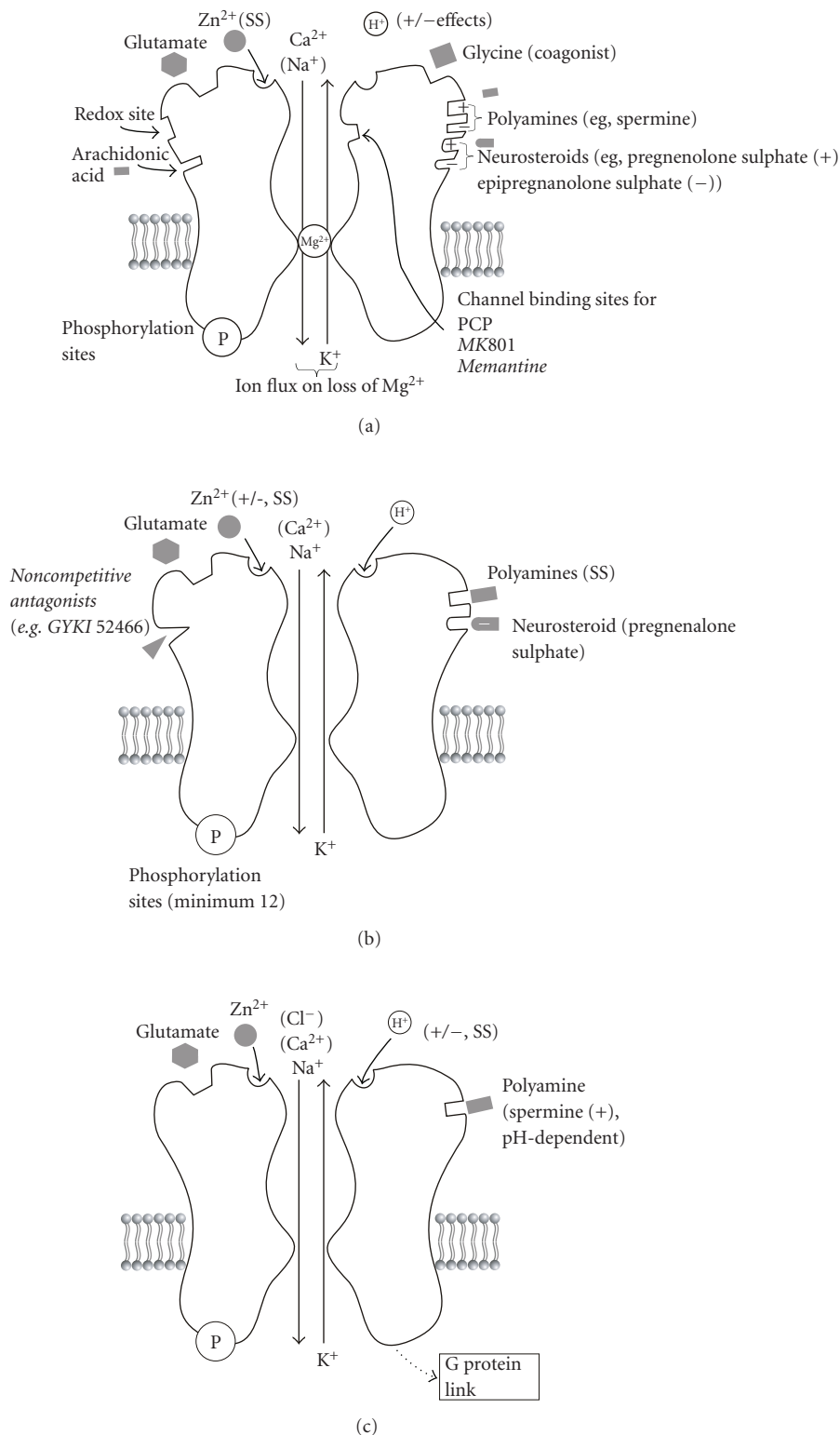
(a)

(b)

(c)

FIGURE 1: Modulation of ionotropic glutamate receptor function. (a) NMDA receptor, (b) AMPA receptor, and (c) kainate receptor. The main endogenous modulatory sites for the glutamate ionotropic receptors are shown and the sites of key exogenous pharmacological agents are in italics. (+) stimulatory/potentiating action, (−) inhibitory action, (SS) subunit-specific action. Additional modifying agents (not shown), where the action has not been specified through a binding site on the receptor, are (a) NO, ethanol, histamine (via polyamine site); (b) arachidonic acid (−), NO; (c) ethanol (−), arachidonic acid (−). The diagram is intended as a summary overview and provides an indication of modulation at these receptors. The discovery of new modulatory agents is ongoing, particularly for the AMPA and kainate receptors, where significantly less is known compared to the NMDA receptor. Abbreviations: H^+, proton; NO, nitric oxide; P, phosphorylation site; PCP, phencyclidine.

mechanism that may, at least in part, reduce the effects of enhanced glutamate levels. However, the need for greater control of the excitotoxic actions of ionotropic receptor agonists is apparent and has not diminished. Indeed, over the past decade much effort has been diverted to identifying compounds that can negate glutamate-mediated neurotoxicity incurred as a consequence of conditions such as stroke and head injury. Results to date have been largely negative and evidence for a neuroprotective role of glutamate antagonists in neurodegenerative diseases is lacking [35]. Similarly, despite efforts to develop compounds that act by altering the metabolism of glutamate, no such drugs have been produced. The rationale is now strong for assessing compounds designed to limit the possible damaging effects of glutamate in diseases such as MS and, in particular, to employ the animal counterpart EAE as the in vivo test system of choice.

THE NMDA RECEPTOR AND ANTAGONISTS

The NMDA receptor is most abundant in the cortex, basal ganglia, and sensory pathways of the nervous system, and has also been identified in a variety of nonneuronal and peripheral locations [36]. In particular, the receptor has been found on the neurovasculature and mast cells derived from the CNS [37–42]. The receptor consists of several subunits, comprising the ubiquitous NR1 subunit and a variety of combinations of NR2A to NR2D and the more recently identified NR3 subunit [39, 43, 44]. Each subunit has 4 membrane domains, an extracellular amino terminal region and an intracellular carboxy group tail. The domains 1, 3, and 4 transverse the membrane and domain 2 appears to form the reentrant loop which lines the ion channel (Figure 2). The channel pore is normally blocked by Mg^{2+} to prevent ion flux but, on appropriate ligand stimulation, membrane depolarisation occurs and the Mg^{2+} blockade is removed to cause a functional opening of the receptor channel (Figure 1(a)).

The NMDA receptor is of particular interest to pharmacologists as there are a number of ligand binding and modulatory sites that offer potential therapeutic targets for control and points of intervention (Figure 1(a)). Functional NMDA receptor complexes are constructs of the NR1 and NR2/NR3 subunits containing the glycine and glutamate recognition sites, respectively [45–47]. Agonists, including NMDA and glutamate, bind to the glutamate recognition site, whereas competitive antagonists such as selfotel may occupy a single region, distinct from the agonist site, but coupled to provide a competitive interaction. Interestingly, selfotel has been effectively used in vivo to block NMDA-induced BBB permeability increases [48].

Glycine and D-serine act as coagonists, through the glycine site, to prevent receptor desensitisation and are prerequisites for the generation of enhanced inward flow of current at the receptor. Histamine and the polyamines (PAs), including spermine and spermidine, act as receptor modulators to both potentiate and inhibit NMDA-induced responses through distinct sites [49–51]. The receptor can also be modulated by sigma site ligands at a position distinct from the channel-blocking site [52]. A clearer understanding of sigma site function in glutamate-mediated responses is required before agents, directed at the target, can be designed to offer therapeutic efficacy. The current extent of NMDA receptor modulatory sites is summarised in Figure 1(a).

NMDA receptors have been extensively studied and show special pharmacological properties that are thought to play a role in pathophysiological mechanisms. For example, the receptor is highly permeable to Ca^{2+} and other cations, including sodium (Na^+) and potassium (K^+), and is readily blocked by physiological concentrations of Mg^{2+} when the cell is normally polarised [9]. The Ca^{2+} permeability of the receptor is controlled by an asparagine residue in the NR1 subunit within the channel pore loop structure of the second membrane domain [53]. The residue also determines the voltage-dependent Mg^{2+} blockade of the NMDA receptor channel [54]. Depolarisation of the receptor leads to loss of Mg^{2+} from the channel pore and an influx of Ca^{2+} with subsequent activation of enzyme systems we, and others, have shown to be pertinent to the inflammatory processes involved in EAE, including NO and PA production [55, 56]. Indeed, Bolton et al first showed elevated NO and PA levels in CNS tissues from EAE-diseased rats prompting the suggestion of an important role for the NMDA receptor in the pathogenesis of the disease and, by implication, in MS [4].

The open channel can be blocked by the uncompetitive NMDA receptor antagonist (+)MK-801 (dizocilpine maleate) (Figures 1(a) and 3), thereby limiting the flow of Ca^{2+} into the cell and curbing activation of enzyme systems [57]. Our subsequent studies using (+)MK-801 confirmed a role for the NMDA receptor in EAE through the prevention of BBB breakdown and neurological deficits and strongly suggests the involvement of glutamate in the disease [58]. A recent investigation by Sharp et al [59] has described the use of (+)MK-801 to confirm NMDA receptor involvement in an in vitro model of BBB damage, and our studies with the drug have indicated the existence of the receptor on immortalised bEnd 3 brain endothelial cells [60]. In addition, Zhu and Liu [61] used (+)MK-801 to attenuate glutamate-induced expression of P-glycoprotein on CNS-derived microvessel endothelial cells and further verify the existence of NMDA receptors on neuroendothelium.

THE CONTROL OF EAE THROUGH LIMITING NMDA RECEPTOR ACTIVATION

The precise mechanism of action for (+)MK-801 in EAE is unclear. In vitro studies by us have shown that the compound does not interfere with mitogen-driven T cell proliferation or affect the inflammatory response made by macrophages (unpublished data). However, ongoing studies examining the downstream Ca^{2+}-dependent events triggered as a result of NMDA receptor activation may offer some insight into the actions of the drug in models of neuroinflammation. Preliminary work has shown that treatment of bEnd 3 cells with (+)MK-801 prevents glutamate-induced release of $ONOO^-$ [62]. Treatment of EAE-sensitised animals with (+)MK-801 also reduces the disease-associated increase in CNS levels of the PA putrescine (Figure 4) [56, 63]. PAs, formed by the
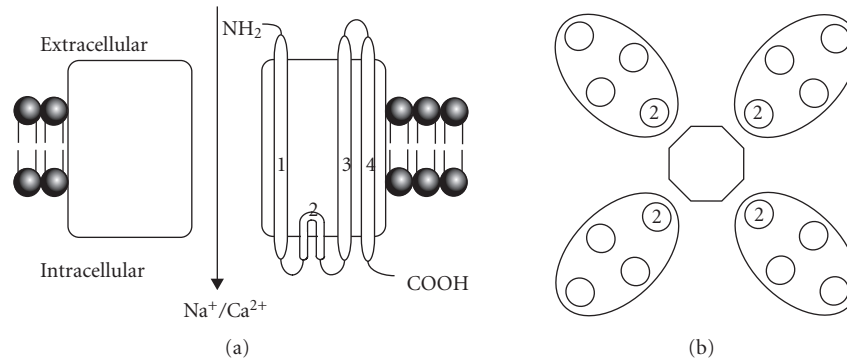
Figure 2: Schematic representation of ionotropic glutamate receptor structure. (a) Each subunit comprises 4 hydrophobic regions of which 1, 3, and 4 are transmembrane domains, while region 2 forms a reentrant loop at the intracellular surface. (b) Receptor subunit organization: the ion channels are formed from four subunits, which orientate allowing the second membrane domain to form the ion channel pore.
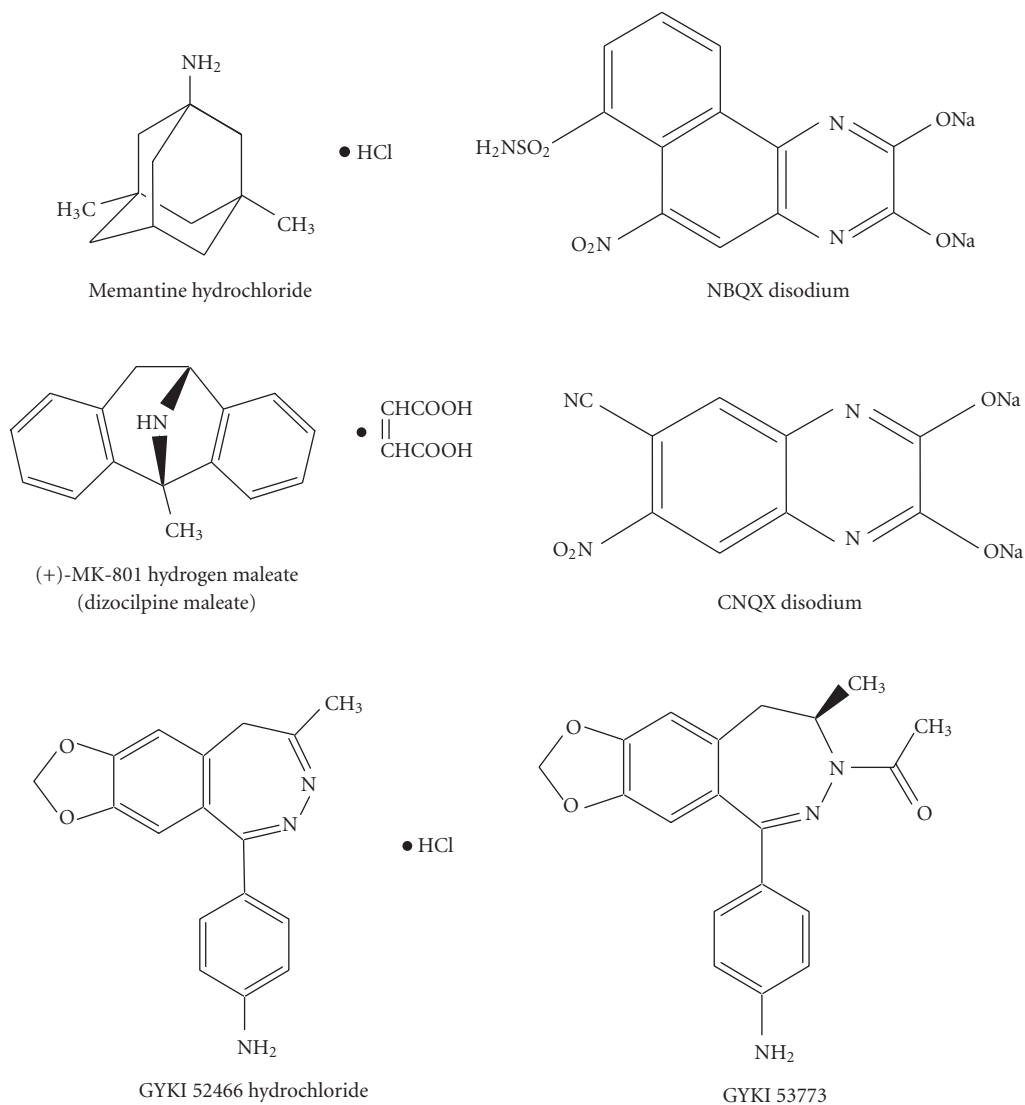

Figure 3: Chemical structures of the main pharmacological agents employed in EAE studies.
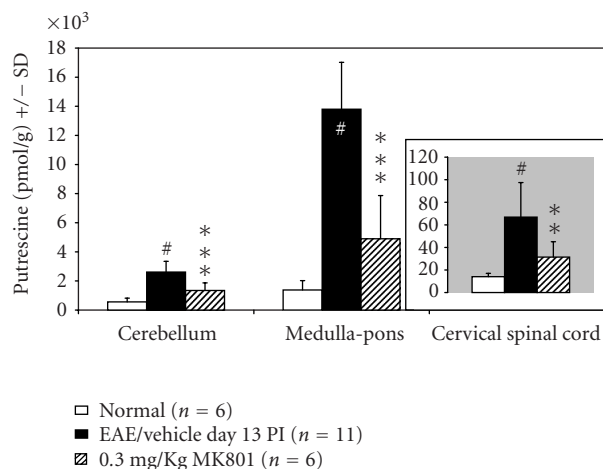
FIGURE 4: Putrescine levels detected in the CNS of normal and acute EAE rats 13 days postinoculation (PI), with and without MK801 treatment. MK801 was administered intraperitoneally for 6 days from day 7 PI at a concentration of 0.3 mg/Kg body weight in sterile phosphate buffered saline. Putrescine levels at day 13 PI are significantly increased in all tissues compared to normal ($\#P < .001$; Student t test). MK801 significantly reduced elevated putrescine at day 13 PI in all CNS areas ($**P < 0.01$, $***P < .001$; Student t test).

rate-limiting Ca^{2+}-dependent enzyme ornithine decarboxylase, act as cell membrane perturbators and vasodisruptors in non-immune-mediated CNS diseases [64, 65]. PAs and $ONOO^-$, along with other ROS, including superoxide and hydroxyl radicals, closely influence neurovascular changes that are typical during the development and progression of EAE and MS.

There is a requirement to clarify PA-mediated events at neurovascular sites with the onset and development of the disease. One approach has been to examine the role of the PAs in EAE by employing enzyme-specific drugs that interrupt the formation of putrescine, spermine, and spermidine, plus compounds that antagonise the PA site on the NMDA receptor. Results indicate a complex series of responses to treatment that are dependent upon the compound, dose, and frequency of administration. Interestingly, the importance of the ornithine decarboxylase-PA pathway in other CNS conditions, including stroke, epilepsy, Alzheimer's disease, and schizophrenia, is being realised and will undoubtedly lead to a determined effort to understand the significance of the agents in disease pathogenesis [66].

Studies by Paul and Bolton [67] together with earlier experiments by Wallstrom et al [68], using the relatively uncompetitive aminoadamantane NMDA receptor antagonist memantine (1-amino-3, 5-dimethyl-adamantane) (Figure 3), confirmed that pharmacological modulation of receptor function during EAE results in disease suppression and restoration of neurovascular function. Importantly, the work by Paul and Bolton indicates, through the use of specific dosing regimes, that NMDA receptor involvement in EAE is at, or just prior to, symptom onset and BBB breakdown, rather than earlier, during the induction phase of disease or later at the height of neurological deficits. Furthermore, a significant effect was noted on neuroinflammatory infiltrates which appears distinct from AMPA/kainite antagonist activity. Memantine, unlike (+)MK-801, has been reported to

differentiate between transient physiological activation and sustained pathological stimulation of the NMDA receptor with actions preferentially directed towards the latter state [69].

The apparent discriminatory profile ascribed to the pharmacology of memantine on abnormal NMDA receptor activity makes the drug particularly attractive for use during the onset of clinical episodes in human CNS diseases. Indeed, memantine has been reported to provide symptomatic relief to MS patients [70]. However, the actual mechanisms through which memantine exerts effects are unclear. One current theory suggests that the compound, like Mg^{2+}, occupies the receptor channel and rapidly exits the pore under strong, physiological synaptic depolarisation and in the presence of glutamate fluxes of millimolar concentrations [71]. In contrast, and under pathological conditions where sustained micromolar concentrations of glutamate preside, memantine, unlike Mg^{2+}, will maintain channel block during prolonged depolarisation.

NMDA receptors play a vital role in maintaining normal synaptic transmission. Consequently, total blockade of the receptor, by compounds including (+)MK-801, leads to numerous side effects. Specific prevention of the pathological activation of NMDA receptors with drugs such as memantine reduces unwanted activity and thereby improves clinical tolerance, offering a useful feature in the treatment of neurodegenerative diseases including MS.

Another approach towards improving specificity and reducing the unwanted side effects of drug therapy might be to target particular modulatory sites on the NMDA receptor. In addition, an alternative to blocking NMDA receptor action completely would be to suppress an exaggerated receptor response. Therefore, targeting inhibitory modulatory sites, such as the PA or neurosteroid binding positions or the poorly defined sigma site (Figure 1(a)), offers the potential to down-regulate rather than completely inhibit NMDA

receptor-mediated events. Alternatively, it may be that the subunit-specific modulatory sites mediate features of neuroinflammatory pathology and thereby become primary targets through which to achieve disease control. Indeed, the NR2B subunit has been recognised as a particular therapeutic target for several neurological conditions [71] and initial studies by Wheeler et al have shown an increased expression of the NR2A and NR2B subunits in CNS tissues from EAE-diseased rats [72, 73].

AMPA and kainate ionotropic glutamate receptors

Postsynaptic AMPA receptors are considered to mediate rapid glutamergic neurotransmission with low Ca^{2+} permeability. The receptor consists of four subunits, GluR1 to GluR4, which are widely distributed throughout the CNS [74, 75] and each of which can be expressed in two variants originally termed "flip" and "flop" (Figure 1(b)). AMPA receptors are invariably colocalised with NMDA receptors indicating a close functional relationship between the 2 ligand-gated cation channel-bearing receptors. Indeed, AMPA activation causes cellular depolarisation and NMDA channel opening with Ca^{2+} influx. Pharmacological studies have provided strong evidence for AMPA receptor involvement in several CNS conditions including stroke, traumatic brain injury, and Parkinson's disease [40].

Kainate receptors are closely related to AMPA receptors and are involved in both pre- and postsynaptic neurotransmission. The receptor class is comprised of 5 subunits falling into 2 families, GluR5 to GluR7 and KA1 plus KA2 (Figure 1(c)). Each subunit family shares 70% sequence homology with its members, but only 40% with nonfamily subunits. Weaker identities are shown with AMPA (30–35%) and NMDA (10–20%) receptor subunits, although some studies have suggested that GluR5 is part of the AMPA family [75–77].

The AMPA and kainate positioning of subunits in their respective receptor complex is similar to the arrangement present in the NMDA receptor. The amino terminal portion of each subunit is extracellular; there are 4 hydrophobic sections, 3 of which are membrane spanning, plus a reentrant membrane loop that contributes to the pore lining. The cytoplasmic carboxy terminus, in common with the NMDA receptor, contains sites for phosphorylation, with a minimum of 12 in the AMPA subunits and a suggested involvement in the regulation of channel function [78, 79]. Fewer modulatory sites have been identified for AMPA and kainate receptors compared to the NMDA receptor (Figures 1(b) and 1(c)). However, information on the endogenous mechanisms for regulating non-NMDA ionotropic receptor function is increasing and a similar capacity for pharmacological modulation of subtype activity can be anticipated.

AMPA/kainate receptors and antagonists in the pathology and control of neuroinflammation

There is scant information on the subtype selectivity of AMPA receptor antagonists (Figure 3) and the standard competitive drugs NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]-quinoxaline-2,3-dione) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) are not selective between non-NMDA ionotropic receptors [80]. NBQX and CNQX have been investigated, with some success, in models of global ischaemia, CNS trauma, and Parkinson's disease [81–83] although drug effects mediated through kainate receptor involvement are suspected and therefore cannot be excluded [84–86].

One cardinal feature of MS and the more chronic models of EAE is the demyelination of central nerve fibres. Restoration of normal nerve function in MS is dependent, at least in part, upon recruitment of myelin-forming oligodendrocytes to lesioned areas. Limited remyelination is possible in acute lesions but virtually nonexistent in chronic states due to lack of oligodendrocyte viability and recruitment to damaged areas [87, 88]. The oligodendrocyte has been reported to express, exclusively, AMPA and kainate receptors, thereby making the cell a target for attack by excitotoxic glutamate in EAE [89–91]. However, investigations by Wosik et al [92] indicate a lack of AMPA receptors on human oligodendrocytes and a resistance to agonist-mediated toxicity. Furthermore, the work suggests that AMPA expression is limited to astrocytes. Despite conjecture over the cellular expression of receptor types in brain tissue, the administration of kainate to the optic nerve causes degenerative toxic lesions, nerve damage in association with inflammation, and demyelination, all of which are strongly suggestive of an MS-related pathology [93]. Interestingly, administration of CNQX prevents kainate-induced lesions whereas the AMPA receptor antagonist GYKI 53655 (1-[4-aminophenyl]-3-methylcarbamyl-7, 8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine) had no significant effect indicating a kainate-specific action and implicating receptor involvement in early MS pathology.

Recent investigations in acute and chronic-relapsing EAE have demonstrated the effectiveness of NBQX together with MPQX ([1,2,3,4-tetrahydro-7-morpholinyl 1,2,3-dioxo-6-(trifluoromethyl)quinoxa lin-1-yl]methylphosphonate) and the noncompetitive antagonists GYKI 52466 (1-(4-aminophenyl) 4-methyl-7,8-methylene dioxy-5H-2,3-benzodiazepine) and GYKI 53773 ((-)1-(4-aminophenyl) 4-methyl-7,8-methylene-dioxy 4,5-dihydro 3-methylcarbamoyl 2,3-benzodiazepine) (Figure 3) in reducing the neurological symptoms of the disease [19, 94, 95]. Interestingly, earlier related work demonstrated that NBQX had anti-oedematous effects at neurovascular sites via a proposed action on glial cells [96]. EAE studies using the competitive antagonists to modify the course of disease cannot exclude drug effects on kainate receptor-mediated events. In contrast, the noncompetitive antagonists do differentiate between the two receptors and therefore indicate a specific AMPA involvement in disease development. More recently, a series of AMPA receptor antagonists, with structures based on 2,3-benzodiazepine, have proved effective in reducing the symptoms and morphological changes associated with EAE [97, 98].

The studies with NBQX have highlighted the ability of competitive receptor antagonists to reduce EAE-mediated neuronal death and oligodendrocyte loss despite

the uncertainty of AMPA or kainate involvement [94, 95]. However, the extent of oligodendrocyte depletion may be dependent on additional endogenous factors. For example, testosterone has been shown to amplify both AMPA- and kainate-induced toxicity to oligodendrocytes in vitro [99], suggesting the existence of a steroidal modulatory site on non-NMDA receptors.

The studies described by Pitt et al [94] and Groom et al [95] also considered the possibility that the competitive and noncompetitive antagonists may operate in EAE through an immunosuppressive action. Results showed that competitive antagonists, such as NBQX, did not affect T-cell proliferation rates or reduce perivascular inflammatory cuffs. However, noncompetitive antagonists did suppress mitogen-induced T cell proliferation, thus offering an alternative explanation for the compounds abilities to modify EAE and indicating AMPA receptor involvement in immune-mediated inflammation. Glutamate excitotoxicity, together with neuroinflammatory factors in both EAE and MS, may be important codeterminants in oligodendrocyte death. For example, inflammatory mediators, such as interleukin 1β and tumour necrosis factor-α (TNF-α) can promote in vitro oligodendrocyte apoptosis and changes in the glutamate buffering system of astrocytes. Moreover, the cytokine-induced effects can be blocked by NBQX and CNQX [100, 101].

Interestingly, research into the regulation of gene expression during EAE has identified a reduction in the important plasma membrane Ca^{2+}ATPase2, necessary for cation homeostasis and expressed exclusively in the grey matter, which occurs coincidently with the development of neurological signs [102]. The studies also found that application of kainate to spinal cord slice cultures significantly lowered the mRNA levels of Ca^{2+}ATPase. Collectively, the results implicate glutamate, particularly via kainate receptors, in the suppression of neuronal plasma membrane Ca^{2+}ATPase 2 and abnormal Ca^{2+} levels. Perhaps of greater significance is the observation that NBQX can suppress alterations in glutamate transporter expression during EAE [16]. The study found protein and mRNA levels of EAAC1 to be dramatically increased, while transporters GLT-1 and GLAST were down-regulated together with a concomitant reduction in the incidence of disease. NBQX, administered semiprophylactically from day 7 postinoculation, suppressed the changes in the expression of transporters suggesting the activation of non-NMDA receptors.

Undoubtedly, the studies indicate an important role for the AMPA receptor in EAE and, possibly, in MS. However, the investigations cannot exclude the possible contribution of kainate receptors in the development of disease pathology. The prospect of AMPA/kainate receptor involvement in experimental and human neuroinflammatory conditions offers new targets to focus treatments for the demyelinating diseases with an emphasis on the preservation of oligodendrocyte function.

SUMMARY

We have reviewed the evidence for ionotropic glutamate receptor involvement in EAE and speculated on a role for the receptors in MS. The finding of both NMDA and non-NMDA receptor involvement in the pathology of EAE is substantiated. Therefore, it is our belief that therapeutic targeting of both receptor groups in models of EAE represents a viable proposition for the development of new treatments for MS.

The observation that a variety of NMDA, AMPA, and kainate receptor antagonists are beneficial in EAE corroborates the considerable involvement of glutamate in the pathology of the disease. Aberrant glutamate transporter mechanisms in resident cells of the CNS together with altered ionotropic receptor or subunit receptor expression during EAE may collectively contribute to excess glutamate levels in target tissues and the gross disturbance to normal homeostasis and nerve function.

Figure 5 summarises the involvement of glutamate in EAE and, by inference MS, highlights the ways through which excitotoxic levels of the amino acid could be achieved. Direct discharge from resident and infiltrating cells or indirect release resulting from the actions of inflammatory mediators would serve to raise CNS glutamate concentrations. The consequences of damage to oligodendrocytes, neurons, and the BBB, plus inflammatory cytokine release from microglia, contribute considerably to the pathology of EAE and could account, at least in part, for some of the major central disturbances observed in MS.

The control of glutamate release and metabolism may offer a viable therapeutic approach to limiting the subsequent damage associated with excitotoxicity. Suppression of agonist-activated ionotropic receptor function has proved effective in controlling EAE, but efficacy in MS remains largely uninvestigated. Regulation of abnormal receptor function rather than total blockade of activity may effectively reduce the results of enhanced CNS glutamate levels and allow homeostatic mechanisms to operate thereby reducing unwanted side effects.

A clear delineation between the receptor type targeted and the ensuing benefits to limit the disease process appears to exist. For example, axonal sparing and oligodendrocyte protection arises from the use of non-NMDA receptor competitive antagonists whereas the restriction of BBB dysfunction and reduction of inflammation can be ascribed to drug effects on the NMDA receptor. However, an exclusive action for the compounds at their respective target sites cannot be guaranteed. Therefore, coadministration of ionotropic receptor antagonists, with different specificities, offers the real prospect of inhibiting several fundamental parameters of experimental and human demyelinating disease. Indeed, a recent study by Kanwar et al [103] has indirectly addressed our suggestion by treating EAE with NBQX in conjunction with a monoclonal antibody directed against mucosal addressin cell adhesion molecule-1 and the N-terminal tripeptide of insulin-like growth factor. Unremitting disease was ameliorated and oligodendrocyte survival and remyelination were increased. Furthermore, CNS inflammation, apoptosis, and axonal damage were reduced. Finally, there is a requirement for an increased selectivity of antagonists towards specific receptor subtypes, either through targeting a specific subunit
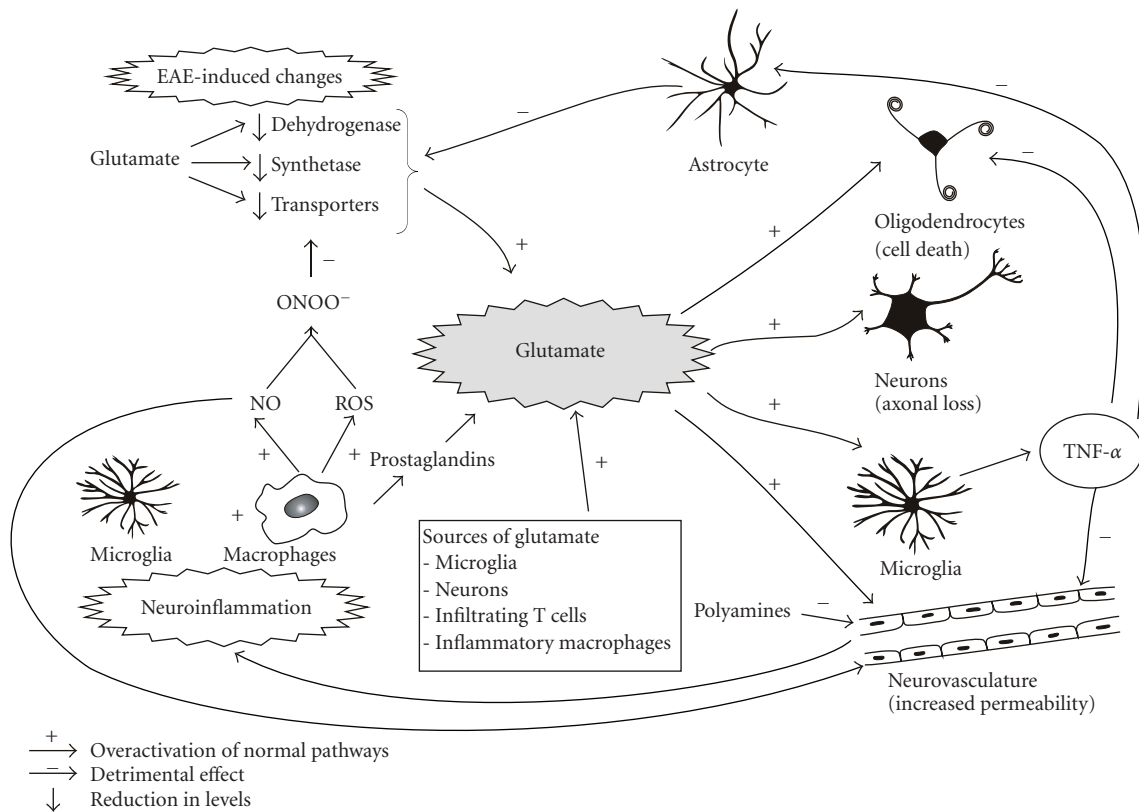
FIGURE 5: Schematic diagram summarising the known and proposed role of glutamate and associated relevant mediators in the development of neuroinflammatory and neurodegenerative pathology during EAE. Abbreviations: EAE, experimental autoimmune encephalomyelitis; NO, nitric oxide; ONOO$^-$, peroxynitrite; ROS, reactive oxygen species; TNF-α, tumour necrosis factor alpha.

or by targeting a modulatory site, if the true therapeutic potential of ionotropic receptor inhibition is to be realised.

A concerted effort to search for drugs with possible efficacy in MS that operate at nonimmunological sites or do not have exclusive, immunosuppressive properties could be viewed as an unconventional approach to disease management. However, compounds designed to antagonise the agonist actions on NMDA and AMPA/kainate receptors administered either alone or in combination with other therapies may offer the real prospect of treatment for patients with MS and related disorders of the CNS.

ACKNOWLEDGMENT

REFERENCES

[1] Waubant E. Emerging disease modifying therapies for multiple sclerosis. *Expert Opinion on Emerging Drugs.* 2003;8 (1):145–161.

[2] 't Hart BA, Amor S. The use of animal models to investigate the pathogenesis of neuroinflammatory disorders of the central nervous system. *Current Opinion in Neurology.* 2003;16 (3):375–383.

[3] Bolton C. Recent advances in the pharmacological control of experimental allergic encephalomyelitis (EAE) and the implications for multiple sclerosis treatment. *Multiple Sclerosis.* 1995;1(3):143–149.

[4] Bolton C, Lees P, Paul C, Scott GS, Williams KI, Woodyer P. Aspects of the biochemical pharmacology of neurovascular disruption in experimental allergic encephalomyelitis (EAE). *Journal of Neuroimmunology.* 1994;52(2):113.

[5] Paul C, Bolton C. Restoration of blood-brain barrier integrity by dexamethasone and cyclosporin A combined dose therapy during experimental allergic encephalomyelitis. *Journal of Neuroimmunology.* 1994;54(1-2):188.

[6] Flanagan EM, Erickson JB, Viveros OH, Chang SY, Reinhard JF Jr. Neurotoxin quinolinic acid is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis. *Journal of Neurochemistry.* 1995;64(3):1192–1196.

[7] Sarchielli P, Greco L, Floridi A, Floridi A, Gallai V. Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. *Archives of Neurology.* 2003;60(8):1082–1088.

[8] Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science.* 1969;164 (880):719–721.

[9] Rang HP, Dale MM, Ritter JM, Moore P. *Pharmacology.* 5th ed. London, UK: Churchill Livingstone; 2003.

[10] Kanai Y, Trotti D, Nussberger S, Hediger MA. The high-affinity glutamate transporter family: structure, function and physiological relevance. In: Reith MEA, ed. *Neurotransmitter*

Transporters: Structure, Function, and Regulation. Totowa, NJ: Humana Press; 1997:171–214.

[11] Plaut GS. Effectiveness of amantadine in reducing relapses in multiple sclerosis. *Journal of the Royal Society of Medicine.* 1987;80(2):91–93.

[12] Stover JF, Pleines UE, Morganti-Kossmann MC, Kossmann T, Lowitzsch K, Kempski OS. Neurotransmitters in cerebrospinal fluid reflect pathological activity. *European Journal of Clinical Investigation.* 1997;27(12):1038–1043.

[13] Klivenyi P, Kekesi K, Juhasz G, Vecsei L. Amino acid concentrations in cerebrospinal fluid of patients with multiple sclerosis. *Acta Neurologica Scandinavica.* 1997;95(2):96–98.

[14] Hardin-Pouzet H, Krakowski M, Bourbonniére L, Didier-Bazes M, Tran E, Owens T. Glutamate metabolism is downregulated in astrocytes during experimental allergic encephalomyelitis. *Glia.* 1997;20(1):79–85.

[15] Rothstein JD. Neurobiology. Bundling up excitement. *Nature.* 2000;407(6801):141–142.

[16] Ohgoh M, Hanada T, Smith T, et al. Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology.* 2002;125(1-2):170–178.

[17] Piani D, Frei K, Do KQ, Cuénod M, Fontana A. Murine brain macrophages induced NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. *Neuroscience Letters.* 1991;133(2):159–162.

[18] Noseworthy JH. Progress in determining the causes and treatment of multiple sclerosis. *Nature.* 1999;399(6738 suppl): A40–A47.

[19] Smith T, Groom A, Zhu B, Turski L. Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nature Medicine.* 2000;6(1):62–66.

[20] Gilgun-Sherki Y, Panet H, Melamed E, Offen D. Riluzole suppresses experimental autoimmune encephalomyelitis: implications for the treatment of multiple sclerosis. *Brain Research.* 2003;989(2):196–204.

[21] Rose JW, Hill KE, Watt HE, Carlson NG. Inflammatory cell expression of cyclooxygenase-2 in the multiple sclerosis lesion. *Journal of Neuroimmunology.* 2004;149(1-2):40–49.

[22] Bolton C, Gordon D, Turk JL. Prostaglandin and thromboxane levels in central nervous system tissues from rats during the induction and development of experimental allergic encephalomyelitis (EAE). *Immunopharmacology.* 1984;7(2): 101–107.

[23] Bolton C, Turner AM, Turk JL. Prostaglandin levels in cerebrospinal fluid from multiple sclerosis patients in remission and relapse. *Journal of Neuroimmunology.* 1984;6(3):151–159.

[24] Bolton C, Parker D, McLeod J, Turk JL. A study of the prostaglandin and thromboxane content of the central nervous tissues with the development of chronic relapsing allergic encephalomyelitis. *Journal of Neuroimmunology.* 1986;10 (3):201–208.

[25] Bezzi P, Carmignoto G, Pasti L, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature.* 1998;391(6664):281–285.

[26] Sanzgiri RP, Araque A, Haydon PG. Prostaglandin E(2) stimulates glutamate receptor-dependent astrocyte neuromodulation in cultured hippocampal cells. *Journal of Neurobiology.* 1999;41(2):221–229.

[27] Salvemini D, Seibert K, Masferrer JL, Settle SL, Currie MG, Needleman P. Nitric oxide activates the cyclooxygenase pathway in inflammation. *American Journal of Therapeutics.* 1995; 2(9):616–619.

[28] Kaufmann WE, Andreasson KI, Isakson PC, Worley PF. Cyclooxygenases and the central nervous system. *Prostaglandins.* 1997;54(3):601–624.

[29] Torreilles J. Nitric oxide: one of the more conserved and widespread signaling molecules. *Frontiers in Bioscience.* 2001; 6:D1161–D1172.

[30] Trotti D, Rossi D, Gjesdal O, et al. Peroxynitrite inhibits glutamate transporter subtypes. *The Journal of Biological Chemistry.* 1996;271(11):5976–5979.

[31] Gurwitz D, Kloog Y. Peroxynitrite generation might explain elevated glutamate and aspartate levels in multiple sclerosis cerebrospinal fluid. *European Journal of Clinical Investigation.* 1998;28(9):760–761.

[32] Touil T, Deloire-Grassin MSA, Vital C, Petry KG, Brochet B. In vivo damage of CNS myelin and axons induced by peroxynitrite. *NeuroReport.* 2001;12(16):3637–3644.

[33] Mattle HP, Lienert C, Greeve I. Uric acid and multiple sclerosis [in German]. *Therapeutische Umschau.* 2004;61(9):553–555.

[34] Schori H, Yoles E, Schwartz M. T-cell-based immunity counteracts the potential toxicity of glutamate in the central nervous system. *Journal of Neuroimmunology.* 2001;119(2):199–204.

[35] Danysz W, Parsons CG. Neuroprotective potential of ionotropic glutamate receptor antagonists. *Neurotoxicity Research.* 2002;4(2):119–126.

[36] Skerry TM, Genever PG. Glutamate signalling in nonneuronal tissues. *Trends in Pharmacological Sciences.* 2001;22 (4):174–181.

[37] Purcell WM, Doyle KM, Westgate C, Atterwill CK. Characterisation of a functional polyamine site on rat mast cells: association with a NMDA receptor macrocomplex. *Journal of Neuroimmunology.* 1996;65(1):49–53.

[38] Koenig H, Trout JJ, Goldstone AD, Lu CY. Capillary NMDA receptors regulate blood-brain barrier function and breakdown. *Brain Research.* 1992;588(2):297–303.

[39] Krizbai IA, Deli MA, Pestenácz A, et al. Expression of glutamate receptors on cultured cerebral endothelial cells. *Journal of Neuroscience Research.* 1998;54(6):814–819.

[40] Parsons CG, Danysz W, Quack G. Glutamate in CNS disorders as a target for drug development: an update. *Drug News & Perspectives.* 1998;11(9):523–569.

[41] Parfenova H, Fedinec A, Leffler CW. Ionotropic glutamate receptors in cerebral microvascular endothelium are functionally linked to heme oxygenase. *Journal of Cerebral Blood Flow & Metabolism.* 2003;23(2):190–197.

[42] Šťastný F, Schwendt M, Lisý V, Jezová D. Main subunits of ionotropic glutamate receptors are expressed in isolated rat brain microvessels. *Neurological Research.* 2002;24(1):93–96.

[43] Sun L, Margolis FL, Shipley MT, Lidow MS. Identification of a long variant of mRNA encoding the NR3 subunit of the NMDA receptor: its regional distribution and developmental expression in the rat brain. *FEBS Letters.* 1998;441(3):392–396.

[44] Nishi M, Hinds H, Lu H-P, Kawata M, Hayashi Y. Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. *The Journal of Neuroscience.* 2001;21(23): RC185.1–RC185.6.

[45] Monyer H, Sprengel R, Schoepfer R, et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science.* 1992;256(5060):1217–1221.

[46] Hirai H, Kirsch J, Laube B, Betz H, Kuhse J. The glycine binding site of the *N*-methyl-D-aspartate receptor subunit NR1:

identification of novel determinants of co-agonist potentiation in the extracellular M3-M4 loop region. *Proceedings of the National Academy of Sciences of the United States of America.* 1996;93(12):6031–6036.

[47] Laube B, Hirai H, Sturgess M, Betz H, Kuhse J. Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate binding site on the NR2B subunit. *Neuron.* 1997;18(3):493–503.

[48] Miller RD, Monsul NT, Vender JR, Lehmann JC. NMDA- and endothelin-1-induced increases in blood-brain barrier permeability quantitated with Lucifer yellow. *Journal of the Neurological Sciences.* 1996;136(1-2):37–40.

[49] Williams K. Subunit-specific potentiation of recombinant *N*-methyl-D-aspartate receptors by histamine. *Molecular Pharmacology.* 1994;46(3):531–541.

[50] Johnson TD. Modulation of channel function by polyamines. *Trends in Pharmacological Sciences.* 1996;17(1):22–27.

[51] Williams K. Modulation and block of ion channels: a new biology of polyamines. *Cellular Signalling.* 1997;9(1):1–13.

[52] Monnet FP, Debonnel G, de Montigny C. The cytochromes P-450 are not involved in the modulation of the *N*-methyl-D-aspartate response by sigma ligands in the rat CA3 dorsal hippocampus. *Synapse.* 1993;13(1):30–38.

[53] Burnashev N, Schoepfer R, Monyer H, et al. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science.* 1992;257(5075): 1415–1419.

[54] Kuner T, Wollmuth LP, Karlin A, Seeburg PH, Sakmann B. Structure of the NMDA receptor channel M2 segment inferred from the accessibility of substituted cysteines. *Neuron.* 1996;17(2):343–352.

[55] Bolton C. Neurovascular damage in experimental allergic encephalomyelitis: a target for pharmacological control. *Mediators of Inflammation.* 1997;6(5-6):295–302.

[56] Paul C, McDonald MC, Seiler N, Bolton C. Altered polyamine (PA) synthesis in the central nervous system (CNS) of experimental allergic encephalomyelitis (EAE)-sensitised rats is linked to blood-brain barrier (BBB) impairment. *Journal of Neuroimmunology.* 1998;90(1):24.

[57] Iversen LL. MK-801 (dizocilpine maleate)-NMDA receptor antagonist. *Neurotransmissions.* 1994;10(1):1–3.

[58] Bolton C, Paul C. MK-801 limits neurovascular dysfunction during experimental allergic encephalomyelitis. *The Journal of Pharmacology and Experimental Therapeutics.* 1997;282 (1):397–402.

[59] Sharp CD, Hines I, Houghton J, et al. Glutamate causes a loss in human cerebral endothelial barrier integrity through activation of NMDA receptor. *American Journal of Physiology - Heart and Circulatory Physiology.* 2003;285(6):H2592–H2598.

[60] Scott GS, Bowman SR, Bolton C. Glutamate induced peroxynitrite production in a brain-derived endothelial cell line is via *N*-methyl-D-aspartate (NMDA) receptor activity. Submitted to *Biochemical Pharmacology.*

[61] Zhu H-J, Liu G-Q. Glutamate up-regulates P-glycoprotein expression in rat brain microvessel endothelial cells by an NMDA receptor-mediated mechanism. *Life Sciences.* 2004;75 (11):1313–1322.

[62] Bowman SR, Scott GS, Bolton C. Glutamate stimulates peroxynitrite production in a brain-derived endothelial cell line via *N*-methyl-D-aspartate (NMDA) receptor activation. *Journal of Neuroimmunology.* 2004;154(1-2):168.

[63] Paul C, Scott GS, Barbour M, Seiler N, Bolton C. *N*-methyl-D-aspartate receptor-mediated events contribute to

neurovascular breakdown during experimental allergic encephalomyelitis (EAE). *Biochemical Society Transactions.* 1997;25(2):167S.

[64] Koenig H, Goldstone AD, Lu CY. Blood brain barrier breakdown in brain edema following cold injury is mediated by microvascular polyamines. *Biochemical and Biophysical Research Communications.* 1983;116(3):1039–1048.

[65] Koenig H, Goldstone AD, Lu CY. Blood-brain barrier breakdown in cold-injured brain is linked to a biphasic stimulation of ornithine decarboxylase activity and polyamine synthesis: both are coordinately inhibited by verapamil, dexamethasone, and aspirin. *Journal of Neurochemistry.* 1989;52(1):101–109.

[66] Bernstein H-G, Müller M. The cellular localization of the L-ornithine decarboxylase/polyamine system in normal and diseased central nervous systems. *Progress in Neurobiology.* 1999;57(5):485–505.

[67] Paul C, Bolton C. Modulation of blood-brain barrier dysfunction and neurological deficits during acute experimental allergic encephalomyelitis by the *N*-methyl-D-aspartate receptor antagonist memantine. *The Journal of Pharmacology and Experimental Therapeutics.* 2002;302(1):50–57.

[68] Wallström E, Diener P, Ljungdahl Å, Khademi M, Nilsson C-G, Olsson T. Memantine abrogates neurological deficits, but not CNS inflammation, in Lewis rat experimental autoimmune encephalomyelitis. *Journal of the Neurological Sciences.* 1996;137(2):89–96.

[69] Parsons CG, Danysz W, Quack G. Memantine is a clinically well tolerated *N*-methyl-D-asparate (NMDA) receptor antagonist—a review of preclinical data. *Neuropharmacology.* 1999;38(6):735–767.

[70] Starck M, Albrecht H, Pollmann W, Straube A, Dieterich M. Drug therapy for acquired pendular nystagmus in multiple sclerosis. *Journal of Neurology.* 1997;244(1):9–16.

[71] Chazot PL. The NMDA receptor NR2B subunit: a valid therapeutic target for multiple CNS pathologies. *Current Medicinal Chemistry.* 2004;11(3):389–396.

[72] Wheeler E, Bolton C, Mullins J, Paul C. Identification of the NMDA receptor subtype involved in blood-brain barrier breakdown during EAE. *Immunology.* 2002;107(suppl. 43–47):46.

[73] Wheeler E, Bolton C, Mullins J, Paul C. Identification of the NMDA receptor subtype involved in the development of disease during experimental autoimmune encephalomyelitis. *British Journal of Pharmacology.* 2003;139:18P.

[74] Martin LJ, Blackstone CD, Levey AI, Huganir RL, Price DL. AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience.* 1993;53(2):327–358.

[75] Huettner JE. Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron.* 1990;5(3):255–266.

[76] Castillo PE, Malenka RC, Nicoll RA. Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature.* 1997;388(6638):182–186.

[77] Lerma J, Morales M, Vicente MA, Herreras O. Glutamate receptors of the kainate type and synaptic transmission. *Trends in Neurosciences.* 1997;20(1):9–12.

[78] Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. *Trends in Neurosciences.* 2002;25(11):578–588.

[79] Huettner JE. Kainate receptors and synaptic transmission. *Progress in Neurobiology.* 2003;70(5):387–407.

[80] Lerma J, Paternain AV, Rodríaguez-Moreno A, López-García JC. Molecular physiology of kainate receptors. *Physiological Reviews.* 2001;81(3):971–998.

[81] Wachtel H, Kunow M, Löschmann P-A. NBQX (6-nitro-sulfamoyl-benzo-quinoxaline-dione) and CPP (3-carboxy-piperazin-propyl phosphonic acid) potentiate dopamine agonist induced rotations in substantia nigra lesioned rats. *Neuroscience Letters.* 1992;142(2):179–182.

[82] Bernert H, Turski L. Traumatic brain damage prevented by the non-*N*-methyl-D-aspartate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f] quinoxaline. *Proceedings of the National Academy of Sciences of the United States of America.* 1996;93(11):5235–5240.

[83] Łazarewicz JW, Gadamski P, Parsons CG, Danysz W. Protection against post-ischaemic neuronal loss in gerbil hippocampal CA1 by glycine B and AMPA antagonists. *Journal of Neural Transmission.* 1997;104(11-12):1249–1254.

[84] Stein E, Cox JA, Seeburg PH, Verdoorn TA. Complex pharmacological properties of recombinant alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor subtypes. *Molecular Pharmacology.* 1992;42(5):864–871.

[85] Fletcher EJ, Nutt SL, Hoo KH, et al. Cloning, expression and pharmacological characterization of a human glutamate receptor: hGluR4. *Receptors and Channels.* 1995;3(1):21–31.

[86] Korczak B, Nutt SL, Fletcher EJ, et al. cDNA cloning and functional properties of human glutamate receptor EAA3 (GluR5) in homomeric and heteromeric configuration. *Receptors and Channels.* 1995;3(1):41–49.

[87] Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho E-S. Multiple sclerosis: remyelination of nascent lesions. *Annals of Neurology.* 1993;33(2):137–151.

[88] Lassmann H. Pathology of multiple sclerosis. In: Compston A, Ebers G, Lassmann H, McDonald I, Matthews B, Wekerle H, eds. *McAlpine's Multiple Sclerosis.* London, UK: Churchill Livingstone; 1998:323–358.

[89] Patneau DK, Wright PW, Winters C, Mayer ML, Gallo V. Glial cells of the oligodendrocyte lineage express both kainate- and AMPA-preferring subtypes of glutamate receptor. *Neuron.* 1994;12(2):357–371.

[90] Yoshioka A, Hardy M, Younkin DP, Grinspan JB, Stern JL, Pleasure D. *α*-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors mediate excitotoxicity in the oligodendroglial lineage. *Journal of Neurochemistry.* 1995; 64(6):2442–2448.

[91] McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg MP. Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. *Nature Medicine.* 1998;4(3):291–297.

[92] Wosik K, Ruffini F, Almazan G, Olivier A, Nalbantoglu J, Antel JP. Resistance of human adult oligodendrocytes to AMPA/kainate receptor-mediated glutamate injury. *Brain: A Journal of Neurology.* 2004;127(12):2636–2648.

[93] Matute C. Characteristics of acute and chronic kainate excitotoxic damage to the optic nerve. *Proceedings of the National Academy of Sciences of the United States of America.* 1998;95(17):10229–10234.

[94] Pitt D, Werner P, Raine CS. Glutamate excitotoxicity in a model of multiple sclerosis. *Nature Medicine.* 2000;6(1):67–70.

[95] Groom AJ, Smith T, Turski L. Multiple sclerosis and glutamate. *Annals of the New York Academy of Sciences.* 2003;993: 229–275.

[96] Westergren I, Johansson BB. Blockade of AMPA receptors reduces brain edema following opening of the blood-brain barrier. *Journal of Cerebral Blood Flow & Metabolism.* 1993;13(4):603–608.

[97] Móricz K, Gigler G, Albert M, et al. Effects of EGIS-8332, an AMPA antagonist, in transient focal ischemia and multiple sclerosis in rats. *Journal of Neuroimmunology.* 2004;154(1-2):86.

[98] Szabó H, Berzsenyi P, Nemét L, Andrási F, Horváth K. Investigations with AMPA antagonist 2,3-benzodiazepines in the experimental autoimmune encephalomyelitis model in rats. *Journal of Neuroimmunology.* 2004;154(1-2):86.

[99] Caruso A, Di Giorgi Gerevini V, Castiglione M, et al. Testosterone amplifies excitotoxic damage of cultured oligodendrocytes. *Journal of Neurochemistry.* 2004;88(5):1179–1185.

[100] Matute C, Alberdi E, Domercq M, Pérez-Cerdá F, Pérez-Samartín A, Sánchez-Gómez MV. The link between excitotoxic oligodendroglial death and demyelinating diseases. *Trends in Neurosciences.* 2001;24(4):224–230.

[101] Takahashi JL, Giuliani F, Power C, Imai Y, Yong VW. Interleukin-1*β* promotes oligodendrocyte death through glutamate excitotoxicity. *Annals of Neurology.* 2003;53(5):588–595.

[102] Nicot A, Ratnakar PV, Ron Y, Chen C-C, Elkabes S. Regulation of gene expression in experimental autoimmune encephalomyelitis indicates early neuronal dysfunction. *Brain: A Journal of Neurology.* 2003;126(2):398–412.

[103] Kanwar JR, Kanwar RK, Krissansen GW. Simultaneous neuroprotection and blockade of inflammation reverses autoimmune encephalomyelitis. *Brain: A Journal of Neurology.* 2004;127(6):1313–1331.