

Neuroprotective Potential of Iontropic Glutamate Receptor Antagonists

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From the therapeutic point of view, the real challenge is not only to improve the symptoms, but to interfere with the pathomechanism of the disease. That is why a considerable interest has recently been devoted to developing glutamate receptors antagonists (mainly of the NMDA type) for acute and chronic neurodegeneration. Developing such a treatment that slows down the progression of the disease is extremely time and costs consuming. At present there is consensus that competitive NMDA receptor antagonists will not find therapeutic applications, in contrast to agents acting at the glycine_B site, or channel blockers. Recently, at least seven glycine_B antagonists (e.g. ACEA 1021, GV-150526, GV-196771A, ZD-9379, MRZ 2/576) and over 10 NMDA channel blockers (e.g. Remacemide, ARL-15896AR, HU-211, ADCI, CNS-5161, Neramexane-MRZ 2/579) have been under development, most of them as neuroprotective agents for acute (stroke, trauma) or chronic insult (e.g. Huntington's or Alzheimer's disease). Several substances selective for NR2B NMDA receptor subtypes such as: eliprodil, CP-101606 and Ro-25-6981 have been claimed to have a good neuroprotective profile. This presentation is an attempt to critically review preclinical and scarce clinical experience in the development of new NMDA receptor antagonists as neuroprotective agents according to the following scheme: rational, preclinical findings in animal models and finally clinical experience if available. The general impression is that NMDA receptor antagonists may find use in chronic type of neurodegeneration while AMPA antagonists seems to show better promise in acute insult.

Keywords: Glutamate; NMDA; AMPA; Neuroprotection; Trauma; Stroke; Alzheimer; AIDS; ALS; Huntington; Parkinson; Stroke

INTRODUCTION TO THE ROLE OF GLUTAMATE IN PHYSIOLOGY AND PATHOLOGY

The neurotransmitter glutamate activates three major types of ionotropic receptors, namely α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) and several types of metabotropic receptors. AMPA receptors are involved in fast glutamatergic neurotransmission (Danysz *et al.*, 1995; Parsons *et al.*, 1998). The AMPA receptor channel is formed out of four known subunits GluR1–GluR4 and is permeable to Na⁺ and K⁺ (complexes lacking GluR2 subunits are also permeable to Ca²⁺). NMDA-sensitive ionotropic glutamate receptors coupled to high conductance cationic channels (Na⁺, K⁺, and Ca²⁺) and are positively modulated by polyamines (spermine and spermidine) and the obligatory co-agonist glycine (glycine_B site). The NMDA channel is blocked in a use- and voltage-dependent manner by Mg²⁺. Such a voltage-dependent blockade practically means that NMDA receptors are only activated following depolarisation of the post-synaptic membrane followed by relief of blockade by Mg²⁺. Because of their high Ca²⁺ permeability and the voltage-dependence of their activation, NMDA receptors are particularly suitable as mediators of synaptic plasticity (e.g. learning and memory) but also, under certain conditions, act as promoters of neurotoxicity (see later). The NMDA receptor channel can also be blocked by exogenous agents such as ketamine, dextromethorphan, memantine, phencyclidine and (+)-5-methyl-10,11-dihydro-5H-

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dibenzocyclohepten-5,10-imine maleate ((+)MK-801). Similarly to AMPA receptors, also NMDA receptors are formed as tetrameric assemblies of two representatives of the NMDAR1 family and two of the NMDAR2 family (NR2A–NR2D).

As indicated above, glutamate is a crucial mediator of many physiological functions, but under certain conditions activation of glutamate receptors will kill neurones. This phenomenon was described for the first time by John Olney and called "excitotoxicity" (Rothman and Olney, 1987). It has been implied that excitotoxicity is involved in many types of acute and chronic insults to the CNS including neurodegenerative disorders (Choi, 1995). Disturbance of glutamate homeostasis probably plays a pivotal role in the execution of pathological changes rather than being the cause itself. Many disease states listed below may be triggered by factors that facilitate the neurotoxic potential of endogenous glutamate such as: energy deficits, free radical formation, etc. (Danysz *et al.*, 1995; Parsons *et al.*, 1998).

IONOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS IN ACUTE INSULT

Stroke

There are a number of microdialysis studies in animals indicating that there is a consistent increase in extracellular glutamate concentration during experimental ischaemia (Benveniste *et al.*, 1984; Globus *et al.*, 1988). In man, there is also an increase in CSF and plasma content of glutamate and glycine in patients with progressive, but not with stable stroke (Castillo *et al.*, 1997).

Considering the shortage of energy in neurones during ischaemia, an increase in extracellular glutamate concentration *per se*, is not necessary to evoke damage through activation of glutamate receptors. Simply other factors may increase neuronal vulnerability to physiological levels of glutamate by, e.g. a decrease of resting membrane potential or intracellular buffering of Ca^{2+} ions. Apart from glutamate, oxidative stress, inflammatory reactions and break-down of the blood–brain barrier may also play a role (Ginsberg, 1995a). In cardiac arrest in man, additionally to immediate cortical damage, there is a delayed (*ca.* 7 days) cell loss in the CA1 hippocampal region (Horn and Schlote, 1992) that can probably be targeted by glutamate antagonists. Interestingly, in the 4-VO global 10 min ischaemia model in rats there is a decrease in expression of GluR2 AMPA receptor subunit expression which makes AMPA receptors more permeable to calcium (Pellegrini-Giampietro *et al.*, 1997).

In general, AMPA receptor antagonists seem to be more active in global ischaemia models, while both NMDA and AMPA receptor antagonists show moderate activity in focal ischaemia (Gill, 1994). In post-ischaemia treatment regimes NMDA receptor antagonists in general show better efficacy in permanent ischaemia models (Ginsberg, 1995a,b; Small and Buchan, 1997).

Several recent clinical trials with glutamate antagonists have consistently failed to show beneficial effects in stroke (Table I). In these studies some CNS related side effects such as agitation, hallucinations, confusion and dizziness were reported. This fits well with the fact that neuroprotective effects of most agents in animal models of stroke can only be expected at high doses of NMDA receptor antagonists producing clear side effects which has often been a severe dose-limiting factor in clinical studies. Glycine_B antagonists initially seemed to be promising since, apart from myorelaxation/ataxia, they are less prone to produce other side. However, a recent clinical trial with GV-150526 also failed to show any benefit (Lees *et al.*, 2000). Similarly, NR2B selective agents have been reported to provide neuroprotection without producing side effects. However, it has recently been found that some of these agents may produce a prolongation of the QT interval in the cardiac action potential due to blockade of HERG potassium channels (Gill *et al.*, 1999). Similarly, NMDA channel blockers produced unfavourable outcome in recent trials in stroke (Table I) (Davis *et al.*, 2000).

To sum up, it seems that there is no evidence for efficacy of NMDA receptor antagonists in stroke as evidenced by clinical trials. This is somewhat surprising considering their good efficacy in animal models. The failure of clinical trials could be related to heterogeneity of patients, too long a time window for initiation of treatment, too low a dose used due to limiting side effects or other factors see (Lodder, 2000). In contrast to NMDA receptor antagonist, some hope remains for AMPA receptor antagonists since several agents are still in clinical trials such as YM-872 and PNQX (see Table I). In the authors opinion, it is more likely that rather an agent with several mechanism of action or combination of several agents will find therapeutic use than a single mechanism based treatment.

CNS Trauma

Following traumatic brain injury in rats there are clearly perturbations of energy homeostasis and a significant increase of extracellular glutamate (microdialysis experiments) (Zauner and Bullock, 1995). There is also evidence for a role of oxidative stress, since transgenic mice over-expressing superoxide dismutase show relative resistance to traumatic

TABLE I Ionotropic glutamate receptor antagonists under development for stroke. In case of "Stage" the developmental status has been indicated, i.e. I–III phase of clinical trials; D: discontinued

Substance	Mechanism of action	Company	Stage	Results
Magnesium	NMDA r. channel blocker	UK Med. Res. Council	III	Not known
Cerestat (CNS 1102)	NMDA r. channel blocker	Cambridge Neuroscience	III, D (?)	Unfavourable risk–benefit ratio
Selfotel (CGS 19755)	NMDA r. competitive antagonist	Novartis	III, D	Unfavourable risk–benefit ratio
Eliprodil	NMDA r. NR2 specific antagonists	Synthelabo	III, D	Side effects (QT)
ACEA 1021 (Licostinel)	NMDA r. glycine _B site antagonist	Co Censys	III, D	Side effects (solubility)
GV-150526	NMDA r. glycine _B site antagonist	Glaxo Wellcome	III, D	Lack of benefit
Remacemide (FPL 12924AA)	NMDA r. channel blocker	ASTRA-Zeneca	IIa	Not known
ARL 15896AR	NMDA r. channel blocker	ASTRA Charmwood	IIa	Not known
HU-211 (Dexanabinol)	NMDA r. channel blocker, other actions	Pharmos	II	Not known
YM-872	AMPA r. competitive antagonist	Yamanouchi	II	Not known
CP-101606	NMDA r. NR2 specific antagonists	Pfizer	II	Not known
PNQX (PD 152247)	AMPA r. competitive antagonist	Parke-Davis+NeuroSearch	I	Not known
ZD 9379	NMDA r. glycine _B site antagonist	Zeneca-	I, D	
CGP-40116	NMDA r. competitive antagonist	Novartis	I, D	

brain injury (Mikawa *et al.*, 1996) and free radicals are known to cause a secondary increase in glutamate levels by inhibition of the uptake (Zauner and Bullock, 1995). An increase in glutamate content has also been observed in patients using brain microdialysis (Kanthan and Shuaib, 1995) or CSF sampling (Palmer *et al.*, 1994). Interestingly, a delayed rise in extracellular levels of glutamate that persisted for several days after traumatic insult has been observed in some patients, and was often connected with bad prognosis (death) (Bullock *et al.*, 1995).

Pretreatment with either NMDA or AMPA receptor antagonist provides neuroprotection in animal models of brain trauma, however in case of post-insult treatment only AMPA receptor antagonists (e.g. NBQX) retain this activity (Faden, 1992; Bernert and Turski, 1996). In infant rats it seems that NMDA receptor antagonists such as (+)MK-801 or CPP are neuroprotective while AMPA antagonists like NBQX are not (Ikonomidou and Turski, 1996). The glutamate release inhibitor, riluzole attenuated fluid percussion cortical injury when applied shortly after the insult (Wahl *et al.*, 1997). On the other hand, in contusive spinal trauma NBQX showed neuroprotective effects both in histological and functional parameters (Wrathall *et al.*, 1992; 1996; 1997), while NMDA receptor antagonists show no persistent beneficial effect on recovery (Agrawal and Fehlings, 1997). In infants, subdural haematoma (common causes of mortality associated with child abuse) (Duhaime *et al.*, 1996) and in a rat model of this insult dextromethorphan and memantine combined with flunarizine were neuroprotective (Kleiser *et al.*, 1995).

Clinical trials with the NMDA channel blocker Cerestat (Table II) were suspended due to lack of effect and safety concerns (SCRIP, 1997). Several other agents are currently undergoing clinical testing of tolerability and/or efficacy, the results of which should be available within the near future (Table II).

IONOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS IN CHRONIC NEURODEGENERATIVE DISEASES

Huntington's Disease

The pattern of neuronal loss in the striatum in Huntington's disease is similar to that obtained after excitotoxic lesions in animal (Schwarcz and Köhler, 1983). Also striatal neurodegeneration produced by mitochondrial toxins (3-NP, malonate inhibitors of complex II–III) producing a similar type of damage is attenuated by lesions of the glutamatergic inputs, the glutamate release inhibitor lamotrigine and/or NMDA receptor antagonists such as MK-801 and memantine (Greene and Greenamyre, 1995; Schulz *et al.*, 1996; Lee *et al.*, 2000). Hence, it is likely that

TABLE II Ionotropic glutamate receptor antagonists under development for traumatic brain injury. In case of "Stage" the developmental status has been indicated, i.e. I–III phase of clinical trials; D: discontinued

Substance	Mechanism of action	Company	Stage	Results
Eliprodil (SL 820715)	NMDA r. NR2 specific antagonist	Synthelabo	III	Side effects (QT) ?
Cerestat (CNS 1102)	NMDA r. channel blocker	Cambridge Neuroscience	III, D	Unfavourable risk–benefit ratio
Selfotel (CGS 19755)	NMDA r. competitive antagonist	Novartis	III, D	Unfavourable risk–benefit ratio
SDZ-EAA-494 (D-CPP-ene)	NMDA r. competitive antagonist	Novartis	III, D	?
ZK 200775	AMPA r. competitive antagonist	Schering Pharmos	II, D	?
HU-211 (Dexanabinol)	NMDA r. channel blocker, other actions	Pharmos	II	Not known
CP-101606	NMDA r. NR2 specific antagonists	Pfizer	II	Not known
ACEA 1021 (Licostinel)	NMDA r. glycine _B site antagonist	Co Censys	I, D	Side effects (solubility)
CGP-40116	NMDA r. competitive antagonist	Novartis	I, D	?

mitochondrial dysfunction evoked by these toxins triggers a chain of reactions including excitotoxicity. In fact, there are data indicating a deficit of mitochondrial complex II–III activity in the brains of Huntington's patients (Browne *et al.*, 1997).

Results of clinical trials of Rochester Huntington's Study Group with Remacemide (NMDA channel blocker +/- Co Q10) are due in the Spring of 2001 (Kieburz, 1999).

Amyotrophic Lateral Sclerosis (ALS)

The pattern of neuronal loss in the spinal cord in ALS patients is similar to that obtained after excitotoxic lesions (kainate) in animals (AMPA receptor involvement) (Hugon *et al.*, 1989). Moreover CSF from ALS patients contains an excitotoxic factor that activates AMPA receptors (Couratier *et al.*, 1993). It has also been reported that there is loss of glutamate uptake (EAAT-2) protein (not mRNA) in the spinal cord and motor cortex (Rothstein *et al.*, 1992). Editing of mRNA for the GluR2 subunit of AMPA receptors is reduced in the ventral grey matter of patients with ALS, which favours higher Ca²⁺ permeability of these channels (Takuma *et al.*, 1999). The answer to the question why this particular population of spinal neurons (motor neurons) is most vulnerable relates to the fact that they do not express calcium binding proteins—functioning as Ca²⁺ buffers in the cytoplasm—such as parvalbumin and calbindin (Shaw and Ince, 1997). All this evidence, makes the excitotoxic hypothesis of neuronal damage in ALS quite convincing.

However, the majority of clinical trial with glutamate antagonists completed to date have not been encouraging. Dextromethorphan (NMDA channel blocker) showed no benefit (Blin *et al.*, 1996; Gredal *et al.*, 1997). Similar negative results were obtained with Lamotrigine (glutamate release inhibitor) (Eisen *et al.*, 1993). In contrast, riluzole (glutamate release inhibitor) has been found to increase survival for several months in a clinical trial involving c.a. 1000 patients (Bensimon *et al.*, 1994; Lacomblez *et al.*, 1996). This agent (Rilutek) has been registered as a neuroprotective treatment for ALS in several countries.

Alzheimer's Disease

Over a decade ago Greenamyre suggested that glutamate might be involved in the pathomechanism of neurodegenerative diseases, like Alzheimer's disease (Greenamyre *et al.*, 1988). Here, just selected pieces of evidence are given. In post-mortem samples from brains of Alzheimer's patients there is a decrease in astroglial glutamate carrier EAA2 in the frontal cortex (Li *et al.*, 1997). *In vitro* constituents of senile plaques stimulate microglia to produce an

unknown neurotoxin having agonistic properties at NMDA receptors (Giulian *et al.*, 1995). Moreover, *in vitro* β -amyloid peptide enhances the toxicity of glutamate (Mattson *et al.*, 1992; Brorson *et al.*, 1995) and augments NMDA receptor mediated transmission (Wu *et al.*, 1995).

So far, memantine is the only NMDA receptor antagonist profiled for neurodegenerative dementia that has been used clinically. Low doses of memantine very effectively attenuate lesions of cholinergic neurones in the NBM in rats produced by direct injection of NMDA (Wenk *et al.*, 1994; 1995). The effective dose produced quite low peak plasma levels $<1 \mu\text{M}$ demonstrating good neuroprotective potential of memantine even at such low, therapeutically relevant doses. Similarly, lesions of the NBM produced by a direct injection of mitochondrial toxin 3-nitropropionic acid (3-NP) are also inhibited by Memantine (Wenk *et al.*, 1996). This indicates that NMDA receptors might be a likely link between different factors contributing to the neuronal insult. It has also been suggested that inflammation might play an important role in neurodegeneration in Alzheimer's disease. Wenk's group (Wenk *et al.*, 1998) using a ChAT assay observed that Memantine infused s.c. by Alzet minipumps prevented the loss of cholinergic neurones in the NBM produced by chronic inflammation through infusion of lipopolysaccharide (bacterial wall component, LPS). Memantine also attenuates hippocampal neuronal damage produced by a direct injection of β -amyloid. (Miguel-Hidalgo *et al.*, 1998).

Thus, memantine is an NMDA receptor antagonist that shows symptomatological improvement in animal models and clinical trials (discussion of this point is beyond the scope of present article) and, on the basis of animal experiments, should provide neuroprotection resulting in the inhibition of disease progression (Parsons *et al.*, 1999; Reisberg, 2000; Ruther *et al.*, 2000). Although some clinical data may suggest such activity, pivotal clinical trials proving such a neuroprotective effect remain to be performed.

HIV Dementia

There are a number of indications that glutamate might be involved in some aspects of AIDS related neurological deficits (Lipton, 1992b). Gp 120 (HIV coat protein) *in vitro* produces toxicity that is attenuated by NMDA receptor antagonists such as MK-801 and memantine (Lipton, 1992a). This toxicity is probably secondary to glutamate release from glial cells rather than a direct agonistic effect of gp 120. In line with this *in vitro* evidence, neurodegeneration in transgenic mice over-expressing gp120 is attenuated by memantine (Toggas *et al.*, 1996). This supports the use of NMDA receptor

antagonists, e.g. memantine in the therapy of HIV infected patients. In fact memantine is currently under clinical trials for inhibition of neurological deficits associated with HIV infection (phase II, NTI+Merz+ Co.).

Parkinson's Disease

There is some evidence that neurodegeneration of dopaminergic pathways of the substantia nigra pars compacta (SNc) in Parkinson's disease involves excitotoxicity (Schmidt *et al.*, 1990; Greenamyre and O'Brien, 1991; Blandini and Greenamyre, 1998). *In vitro*, MPTP application (its metabolite MPP⁺ is a mitochondrial complex I inhibitor) inhibits the astroglial glutamate transporter (Hazell *et al.*, 1997), probably through free radicals. In rats, NMDA receptor antagonists protect against damage of dopaminergic neurones induced by the dopaminomimetic methamphetamine (Sonsalla *et al.*, 1991). MPTP-induced toxicity in monkeys is prevented either by NMDA receptor antagonists or by lesion of the descending cortico-striatal glutamatergic pathway (Zuddas *et al.*, 1992; Srivastava *et al.*, 1993; Benazzouz *et al.*, 1995; Bezard *et al.*, 1998). Similarly, in rats, damage to the SNc produced by a direct injection of MPP⁺ in this structure is attenuated by NMDA receptor antagonists (Turski *et al.*, 1991). Interestingly, there is evidence that over-activity of the subthalamic nucleus may contribute to the metabolic compromise in the nigro-striatal system and, in turn, to neuronal loss as lesions of this nucleus were shown to be protective in rats model bearing SNc lesions (Blandini *et al.*, 1997).

Based on the above data one could speculate that NMDA receptor antagonists (and may be AMPA receptor antagonists) should provide some degree of neuroprotection in Parkinson's patients. There is only one clinical study addressing this question which shows that amantadine treatment is an independent predictor of improved survival in Parkinson's disease (Uitti *et al.*, 1996).

CONCLUSIONS

1. Results with NMDA receptor antagonists in clinical trials of stroke and trauma have been very disappointing. Some degree of hope still remains for AMPA and/or kainate receptor antagonists.
2. Clinical evidence for neuroprotective activity of glutamate antagonists in chronic neurodegenerative diseases is scarce. This is probably due to the fact that clinical studies showing this type of activity should be very long lasting and would therefore be extremely costly.

3. So far there are promising results in ALS only with riluzole, and even then, the increase of survival obtained is very modest.
4. Clinical trials with memantine in HIV dementia and remacemide in Huntington's disease are ongoing (results due on the Spring of 2001).
5. Pivotal clinical trials for most indications (Alzheimer's disease, Parkinson's disease and many others) are still missing.
6. "Easy" for testing, fast progressing diseases are the first that are/will be targeted (ALS, Huntington's disease, AIDS dementia).

Moreover, it should be considered that the major goal in the treatment of neurodegenerative diseases should be inhibition of disease progression starting very early, i.e. combined early, sensitive diagnostics. This way neuroprotective therapy would prolong the stage at which patients are professionally and socially active.

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